MODERN TRENDS

IN

ENDOCRINOLOGY

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MODERN TRENDS
IN
ENDOCRINOLOGY

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PREFACE

A VAST AMOUNT of research has gone into endocrinology one of the results being the massive output of literature on the subject It is generally agreed that it is virtually impossible for any busy worker to keep abreast of latest developments and there is therefore a great need for the volume which presents the broadest outlook emphasizes the modern approach and relegates the trite and well worn theories

The authors of the chapters are all experts in their particular fields and their objective has been not only to present the up to date approach but also to give their personal views with a tie up of loose ends and with a critical evaluation of the trend of events possibly pointing to the future The chapters are more there fore than a routine and stereotyped summary of the present position In general the aim has been to highlight the advances in a particular field and to bring the present position into perspective

Particular fields of advance in recent years have been associated with the adrenal hormones and a number of chapters deal with this subject New work on the thyroid hormones forms the opening chapters and the subject of radioactive iodine and its uses in thyroid disease is dealt with extensively The female sex hormones are the subject of later chapters and a comprehensive article on hormonal factors in breast development and milk secretion has been included as has one on human infertility in the female In the study of endocrinology the interaction and inter relationship of hormones can lead the reader from one subject to another with little difficulty As Editor it has been my responsibility to present a compact volume The choice of subjects has been an arbitrary one and the scope of the work is not intended to be comprehensive To decide which subjects must await a possible second series has proved to be a most difficult problem and as the anterior pituitary hormones are considered separately in various papers it was decided that an over all review should not be included This also applies to the parathyroid glands and to the subject of infertility in the male

Briefly this volume has covered many interesting and valuable additions to endocrine knowledge acquired in the last decade and it will be a helpful addition to the library of physicians endocrinologists postgraduates and others whose work necessitates an up-to date knowledge of modern trends in endocrinology

I would like to take this opportunity of expressing my cordial thanks to all those who have contributed to the present volume

H GARDINER HILL

London
October 1957

CHAPTER 1

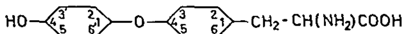
PRESENT KNOWLEDGE OF THE THYROID HORMONES

ROSALIND PITT RIVERS

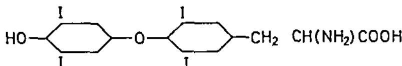
INTRODUCTION

THE PRESENT survey of our knowledge of the thyroid hormones is not intended to be comprehensive Limitations of space make it impossible to deal adequately with the great volume of work which has been done in this field in the past 20 years only the more salient biochemical and physiological discoveries will be considered here

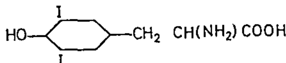
The first advance in our knowledge of the active principle of the thyroid came with Kendall s (1919) isolation of pure thyroxine from pigs thyroid The second advance came with the identification of thyroxine by Harington by degradation and synthesis (Harington 1926 Harington and Barger 1927) Desiodothyroxine (thyronine) was at first shown to be β [4 (4 hydroxyphenoxy)phenyl] alanine



In thyroxine the iodine atoms were shown to occupy the 3 5 and 3 5 positions



Harington (1933) then showed that natural thyroxine possessed the *L* configuration and was structurally related to *L* tyrosine Diiodotyrosine



and iodide were also shown to be present in the thyroid and together with thyroxine were thought to account for all the thyroidal iodine

A NEW THYROID HORMONE

Triiodothyronine

In recent years two new techniques chromatography and autoradiography of compounds labelled with the radioactive isotope of iodine ^{131}I have made a great contribution to our knowledge of thyroid metabolism The former enables us to separate minute amounts of material the latter to locate iodinated products on

chromatograms (Gross 1954) Using these methods Fink and Fink (1948) first demonstrated the presence of 3 monoiodotyrosine in rat thyroid hydrolysates Later Gross *et al* (1950) and Gross and Leblond (1951) showed that the administration of ^{131}I to iodine deficient rats gave rise to the presence of hitherto unknown compounds in their thyroids sera and other tissues One of these compounds (Unknown 1) was demonstrated by Gross and Pitt Rivers (1951) in the plasma of patients who had received therapeutic doses of ^{131}I These authors (Gross and Pitt Rivers *1952a*) identified Unknown 1 as 3 5 3 triiodothyronine and at the same time Roche *et al* (1952) found triiodothyronine among the products of thyroglobulin hydrolysates Gross and Pitt Rivers (1952b) investigated the physiological activity of triiodothyronine in preventing thiouracil induced goitre in rats and Gross Leblond and Trotter (1952) showed that it was highly effective in alleviating myxoedema in man The high biological potency of triiodothyronine which will be described in detail later has made it generally accepted as one of the thyroid hormones

BIOSYNTHESIS

The biosynthesis of the thyroid hormones may be divided into two main stages (1) the concentration of iodide from the circulation and (2) the organic binding of the accumulated iodine

Iodide concentrating mechanism

The iodide concentrating mechanism or iodide trap has been the subject of numerous experiments especially in the last few years The separation of the two biochemical stages for individual study has been made possible by the discovery of two types of antithyroid drugs (*a*) thiocyanate and certain inorganic cations notably perchlorate which inhibit the iodide trap and (*b*) the thiocarbamides (thiouracils aminothiazoles) *which inhibit the organic incorporation* of iodine By administering the thiocarbamides the iodide trap may be investigated independently

It must of course be realized that separation of one stage of hormone synthesis from another may result in abnormal function Nevertheless this method of investigation has enabled workers to study effects of hypophysectomy iodine deficiency or excess and other factors in the stages of thyroid biochemistry Many *in vivo* studies have been carried out notably by Chaikoff (*see* Chaikoff and Taurog 1948 Taurog *et al* 1947 Vanderlaan and Vanderlaan 1947 Vanderlaan and Greer 1950 and Halmi *et al* 1953 1954) *In vitro* studies have been carried out by Chaikoff and more recently by Wyngaarden *et al* (1951)

Notwithstanding the mechanism whereby the thyroid concentrates iodine is still unknown as are the enzyme systems which produce the energy required for this concentration

Organic binding of accumulated iodine

Organic binding of iodine is generally thought to involve the following reactions

(1) Oxidation of iodide to iodine
(2) Iodination of tyrosine to monoiodotyrosine and diiodotyrosine
(3) Oxidative coupling of diiodotyrosine to thyroxine
(4) Formation of triiodothyronine

There are two possibilities partial de iodination of thyroxine or the coupling of one molecule each of monoiodotyrosine and diiodotyrosine no direct evidence in favour of either reaction exists but as Roche and Michel have been unable to demonstrate any de iodination of thyroxine by thyroid slices *in vitro* (though monoiodotyrosine and diiodotyrosine do undergo de iodination) they therefore favour the latter possibility

Chaikoff and his colleagues have been responsible for much enlightenment on the nature of thyroid hormone synthesis (Chaikoff and Taurog 1948) as have Leblond Gross and their group of workers (Gross and Pitt Rivers 1952c Gross 1954) We are however without any real knowledge of the enzymes which oxidize iodide to iodine and those which potentiate the coupling of diiodotyrosine to thyroxine apart from the histochemical evidence for a thyroid peroxidase (Dempsey 1944) Other enzymes in the thyroid have been detected by this means including a pro tease (De Robertis and Nowinski 1946) The thyroid protease has recently been isolated and purified (McQuillan and Trikojus 1953 McQuillan *et al* 1954) and conditions governing its action on thyroglobulin determined This enzyme is responsible for the release of the iodinated amino acids which are mainly present in the gland incorporated into the storage protein thyroglobulin

Small amounts of free iodinated compounds have been detected after the adminis tration of ^{131}I to animals (Gross 1954) these may consist of an equilibrium mixture of iodinated amino acids about to become incorporated into thyroglobulin and those about to pass into the circulation

At present there is no evidence as to whether tyrosine is iodinated in the free state and then bound in protein linkage or whether it is iodinated in the bound state but both theories have their advocates

THE CIRCULATING THYROID HORMONE

The nature of the circulating thyroid hormone was for many years a matter of debate Trevorrow (1939) first suggested that thyroxine itself was the circulating hormone and that it was in some way bound to protein but at the time her work was largely ignored Later Lerman (1940) produced immunological evidence that thyroglobulin was not the circulating hormone It was not until 1948 that Taurog and Chaikoff produced convincing evidence that thyroxine was the principal hormonal constituent in blood a fact which is now established (Laidlaw 1949 Rall 1950 Gross *et al* 1950 Rosenberg 1951) That thyroxine is loosely bound to a protein in the blood (Gordon *et al* 1952 Larson *et al* 1952 Robbins and Rall 1952 and Petermann *et al* 1954) has also been demon strated but as yet this protein has not been identified

The thyroxine building protein migrates in an electrophoretic field between α_1 and α_2 globulins and possesses properties similar to those of Schmids α -glycoprotein Changes in the thyroxine binding pattern in pregnancy and in certain pathological conditions have been studied (Peters *et al* 1948 Danowski *et al* 1950 Horst and Rosler 1953 Horst 1954 and Dowling *et al* 1956)

Abnormal blood iodine components have been detected but their physiological significance is not known while abnormal proteins have been found in the blood after large therapeutic doses of ^{131}I Triiodothyronine also circulates in the blood

though in relatively small amounts and it is associated with protein its binding is however not nearly so specific as that of thyroxine (Deiss *et al* 1953)

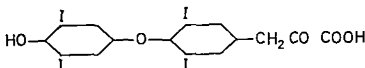
Monoiodotyrosine diiodotyrosine and thyroglobulin are not found in normal sera but they do appear after massive therapeutic doses of ^{131}I and in certain patients with congenital goitre (Stanbury *et al* 1955 1956) further diiodotyrosine has been demonstrated in the urine of nephrotic subjects (Rasmussen 1956) the origin of this diiodotyrosine is not known but it probably appears as a metabolite of thyroid hormone rather than from the thyroid gland via the circulation

METABOLISM

Quantitative aspects of iodine metabolism in man under normal and pathological conditions have been investigated by workers too numerous to mention individually In the present article only the biochemical nature of thyroid metabolites will be considered

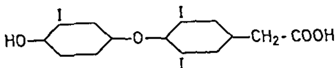
For detailed study the reader is referred to the review by Riggs (1952) later works include the study on endemic goitre by Stanbury *et al* (1954) and papers by Berson and Yalow (1954) Ingbar and Freinkel (1955) and Sterling and Chodos (1956)

As has been said before monoiodotyrosine and diiodotyrosine do not appear in the blood in normal subjects their metabolism is entirely intrathyroidal After proteolytic release from thyroglobulin (but *not* before) they are de iodinated by the dehalogenase of the gland The fate of ^{131}I labelled thyroxine and triiodo thyronine has been studied in patients and in laboratory animals (Gross and Leblond 1947 Albert and Keating 1952 Taurog *et al* 1952 Briggs *et al* 1953 Roche *et al* 1954 and Myant 1956) After injection thyroxine is rapidly distributed between the blood liver and tissues it then passes into the gastro intestinal tract via the bile In the liver both thyroxine and to a lesser extent triiodothyronine are present as conjugates probably glucuronides these glucuronides have not been synthesized but treatment of the conjugates with β glucuronidase leads to the regeneration of the parent amino acids The formation of these glucuronides can be inhibited by liver damage induced by allyl formate intoxication (Beraud *et al* 1956) There is also evidence obtained from a number of colour reactions of carbonyl compounds that bile contains carbonyl derivatives of thyroid hormones Roche *et al* (1954) identified these compounds as pyruvic acid derivatives of thyroxine and triiodothyronine



In rats a considerable amount of iodine is excreted in the faeces (up to 60 per cent) mainly as thyroxine but in man the most important identifiable metabolite of the thyroid hormones is the iodide found in the urine this of course only applies to man in a healthy state Brief mention has already been made of abnormal metabolites found in certain pathological conditions

Triiodothyroacetic acid—Roche and his colleagues have recently provided evidence of the presence of triiodothyroacetic acid (Pitt Rivers 1953) in kidney homo-



genates of rats which had received doses of ^{131}I labelled triiodothyronine this compound could well be derived from the keto acid analogue of triiodothyronine So far however it has not been found in tissues after administration of radio iodide and the possibility exists that the acetic and pyruvic acid analogues do not represent the normal metabolic pathway of thyroid hormones but arise from the body s inability to deal with greater than physiological doses of drugs These findings await confirmation

Conversion of thyroxine to triiodothyronine

The conversion of thyroxine to triiodothyronine *in vivo* has been demonstrated in the mouse (Gross and Leblond 1951) to a slight extent in athyreotic man (Pitt Rivers Stanbury and Rapp 1955) and in the rat (Hogness *et al* 1955) It may well be that the conversion of thyroxine to triiodothyronine is not an essential step in thyroid hormone metabolism the original hypothesis of Gross and Pitt Rivers (1953) that thyroxine was a precursor of triiodothyronine has not been justified by subsequent findings

Albright Larson and Tust (1954) found that rat kidney slices can consistently con vert thyroxine to triiodothyronine *in vitro* Barker on the other hand (personal communication) has only had very occasional evidence of de iodination *in vitro* Sprott and Maclagan (1954) have also obtained de iodination of thyroxine by rat liver homogenates with some chromatographic evidence that triiodothyronine was formed

The presence of diiodotyrosine in invertebrates (corals sponges) has been recognized for many years and recent work on the metabolism of ^{131}I in inverte brates has revealed in one instance the biosynthesis of considerable amounts of thyroxine

Gorbman *et al* (1954) found that after 24 hours immersion in a solution containing ^{131}I the bivalve *musculium partumeium* had concentrated over 40 per cent of the environ mental iodide and had converted as much as 20 per cent of it to thyroxine Whether triiodothyronine was also present could not be determined because the solvent used for chromatographic analysis (butanol acetic acid) does not separate thyroxine and triiodothyronine Roche *et al* (1951) detected thyroxine but only in traces in the gorgonine *Eunicella verrucosa* Pallas though monoiodotyrosine and diiodotyrosine were present in considerable amounts

The formation of thyroxine in invertebrates is of great interest since it demon strates that diiodotyrosine coupling enzymes must exist in nature in the absence of the thyroid gland From the evolutionary standpoint thyroxine biosynthesis has preceded the development of the organ specialized for its production

PHYSIOLOGICAL ACTIVITY

Effects *in vivo*

Myxoedema in man

Although the absence of thyroid function in man and in animals results in myxoedema cretinism and stunted growth these conditions can be overcome by the administration of desiccated thyroid or thyroxine Triiodothyronine has been shown to have qualitatively at least a thyroxine like effect in myxoedematous subjects Gross *et al* (1952) showed that small daily amounts of triiodothyronine (80 micrograms) restored the basal metabolic rate serum cholesterol and weight to normal levels in two patients Since then many workers have studied the effect of triiodothyronine in man Rawson *et al* (1953) and Blackburn *et al* (1954) considered the potencies of thyroxine and triiodothyronine to be the same while Deltour and Bekaert (1953) Lerman (1953) and Asper *et al* (1953) found triiodo thyronine to have a much greater initial activity as much as 5–10 times that of thyroxine Although Deltour and Bekaert (1953) found no difference in their latent period of action it is now generally agreed that in man triiodothyronine has a very rapid effect—changes in temperature and pulse rate may be detected an hour after administration of the drug—but these same effects would take days rather than hours to be obtained with thyroxine

The very rapid relapse of myxoedema patients after withdrawal of triiodothyronine has been noted by Frawley *et al* (1956) who also discerned some side effects (headache tachycardia angina) It was not however, suggested that triiodo thyronine was more likely to produce these symptoms than other thyroid preparations

Kurland *et al* (1955) described a response to triiodothyronine in hypometabolic (non myxoedematous) patients who had not responded to treatment with desic cated thyroid Striking clinical improvement was obtained in some cases

Starr and Liebhold Schueck (1953) have studied the relative effects of thyroxine and triiodothyronine in normal subjects especially with regard to their depressant action on ^{131}I uptake by the thyroid

In laboratory animals

Triiodothyronine is able to raise oxygen consumption in intact rats Gemmill (1953) Heming and Holtkamp (1953a) and Maclagan *et al* (1952) all found a potency of 1–2 in favour of the drug while Colville and Bonnycastle (1953) and Tomich and Woollett (1953) gave the higher ratios of 3–5 in its favour In thyroid ectomized rats the differences were more striking and most workers have found that triiodothyronine shows a consistently higher potency under these conditions Donhoffer (1956) has recently shown that the drug has an immediate action on the basal metabolic rate of hypophysectomized rats

Goitre prevention assay

The discovery of antithyroid drugs of the thiouracil type provided us with a new assay of thyroidal activity Dempsey and Astwood (1943) showed that goitres produced in rats by feeding thiouracil could be reversed by simultaneous adminis tration of thyroxine the effect was proportional to the dose of thyroxine In this assay triiodothyronine was found to be from 3 5 to 7 4 times more active than

thyroxine (Gross and Pitt Rivers 1952b 1953 Tomich and Woollett 1953 Heming and Holtkamp 1953b and Colville and Bonnycastle 1953) Ferguson and Watson (1953) emphasized the importance of the route of administration of these compounds

Mouse anoxia test

The administration of thyroid preparations has been shown to accelerate the death of mice from anoxia (Smith *et al* 1947) Tomich and Woollett (1953) found that triiodothyronine was 4 5 times as active as thyroxine in this test but Gemmill (1953) found only slight differences in potency while Anderson (1954) obtained values in agreement with those of Tomich and Woollett The thyroid stimulating hormone of the pituitary accelerates both the uptake of iodide by the thyroid and the discharge of hormone from the gland, though Wolff (1951) found that this discharge was inhibited in rats by small doses of thyroxine Gilliland and Strudwick (1953) showed that triiodothyronine was more effective than thyroxine in suppressing the discharge of iodine from chicks thyroids—a finding confirmed by Anderson (1954)

Amphibian metamorphosis test

Thyroid substance and thyroxine were early shown to accelerate metamorphosis in amphibia and this effect has been widely used for assay of thyroid like compounds Roth (1953) found only a relatively low potency for triiodothyronine in his first assays but later (Roth 1954) obtained values similar to those of Shellabarger and Godwin (1954) and of Bruice *et al* (1954) the potency of triiodothyronine was 3–4 times that of thyroxine in this test

Miscellaneous effects

Bartlett *et al* (1954) investigated the galactopoietic effects of *L* thyroxine and *l* triiodothyronine in lactating cows and found that when both compounds were given orally 64 milligrams of triiodothyronine daily had much less activity than 75 grammes of thyroxine When the compounds were injected subcutaneously daily doses of 5 milligrams of triiodothyronine were somewhat more effective than 5 milligrams of thyroxine it appears that the method of dosing is important—the lower oral activity of triiodothyronine may be due to its greater solubility and destruction by bacteria in the rumen Triiodothyronine was more active than thyroxine in increasing the heart rate of cows its galactopoietic activity apparently does not parallel its other metabolic effects even when administered by the most advantageous route

Tusques (1953) has shown that 3 daily intraperitoneal injections of thyroxine or of triiodothyronine in newborn rats shortly after birth will induce opening of the eyes on the ninth or tenth day whereas spontaneous opening does not occur until the fourteenth day Maturation of the lachrymal glands opening of the external auditory orifice and other processes were also hastened The two compounds had the same potency and the same latent period of action

Triiodothyronine has also been shown (Bruce *et al* 1954) to reverse the effects of hypothyroidism on the plumage of birds treated with radioactive iodine Thyroxine has long been known to effect this reversal (Parkes and Selye 1937) but

it was not compared with triiodothyronine in this experiment which was designed to test the potency of another triiodothyronine analogue

Effects *in vitro*

An enormous amount of work has been done on the effects of thyroid hormones on isolated tissues and enzyme systems but a survey of this work notably in the reviews by Barker (1951) and Dutoit (1952) shows how little agreement there is between workers and how little we know about the mode of action of these hormones

Another aspect of thyroid hormone action relates to its effect on biological oxidations at a subcellular level It is now generally accepted that such oxidations are coupled with organic binding of inorganic phosphate the energy produced being stored in high energy phosphate bonds

Uncoupling effect —Loomis and Lipmann (1948) first showed that 2 4-dinitrophenol could uncouple phosphorylation and respiration in kidney homogenate though they were unable to obtain this effect with thyroxine Lardy and Feldott (1951) and Lardy and Maley (1954) succeeded in demonstrating the uncoupling effect with both thyroxine and triiodothyronine in rat kidney and liver mitochondria using a wide variety of substrates These authors showed that enzyme preparations from the livers of hyperthyroid rats uncoupled phosphorylation and increased respiration Lipmann and his co workers (Niemeyer *et al* 1951 1952) obtained uncoupling effects from tissues of thyroxinized animals as did Lipner and Barker (1953) and Hoch and Lipmann (1953) Judah (1951) did not obtain any great uncoupling activity from liver mitochrondria of animals pretreated with thyroxine Hoch and Lipmann obtained effects *in vitro* after a short incubation of the mitochondria with thyroxine Klemperer (1955) noted the necessity of a preliminary incubation in order to get a response from thyroxine and triiodothyronine on uncoupling in mitochondria Barker and Klitgaard (1952) showed that certain tissues excised from thyroxinized animals show an increased oxygen consumption in glucose and a lowered oxygen consumption after thyroidectomy These findings have been confirmed by Hoexter (1954) and extended to triiodothyronine by Barker (1955) and Wiswell *et al* (1954) the latter workers were unable to demonstrate any effect on tissue respiration when thyroxine or triiodothyronine were added to tissues *in vitro*

Gemmill (1952a and b) found that thyroxine increases the oxidation of succinate by rat heart homogenate the stimulation being proportional to the amount of thyroxine added triiodothyronine (Gemmill 1953a) has a comparable effect Wiswell *et al* (1954) showed that both these amino acids augment oxygen consumption of heart homogenate in succinate with added cytochrome C thyroglobulin affected this system similarly (Gemmill 1954)

Thyroxine (Fell and Mellanby 1955) stimulated embryonic bone growth in tissue culture (16 micrograms per 100 millilitres medium) as did triiodothyronine (Fell and Mellanby 1956) The low concentration of thyroxine used is noteworthy this is not much greater than what might be expected in a physiological range

Thyroxine has been shown to influence certain systems by removing metals from the medium by complex formation Gemmill (1951a and b) studied its effect on the oxidation of ascorbic acid by the copper enzyme ascorbic acid oxidase from squash (Dunn and Dawson 1951) and also its oxidation catalysed by cupric ions he found that the enzymic oxidation was accelerated and that the copper catalysed oxidation was inhibited Gemmill (1953b) and Gemmill and Plunkett (1952) considered that thyroxine acted by removing the copper as an insoluble complex composed of one mole of cupric chloride

and three moles of thyroxine Frieden (1952) suggested that activation of ascorbic acid oxidase may be due to complexing of thyroxine with copper when he showed that thyroxine reversed the inhibition of the enzyme by cupric ions Mercury zinc and nickel also inhibited the enzyme but to a lesser extent

Askonas (1951) showed that the activity of a partially purified preparation of creatine phosphokinase was inhibited by addition of thyroxine in concentrations between 1-14 × 10^{-4}M enzyme preparations from animals which had received injections of thyroxine also exhibited reduced activity Kuby *et al* (1954a) found that inhibition of crystalline creatine transphosphorylase from rabbit muscle (Kuby *et al* 1954b) by thyroxine was due to the removal of magnesium on which the enzyme is dependent by complex formation Triiodothyronine which did not form insoluble precipitates with magnesium in the concentrations tested had only slight inhibitory activity towards the enzyme

Bain (1954) briefly reported that thyroxine inhibition of oxidative phosphorylation by mitochondria from various tissues could be related to the removal of magnesium as a thyroxine complex The uncoupling effect of thyroxine was enhanced when the concentration of magnesium was below 12 micro moles per millilitre and was reversed by magnesium concentrations above this level The thyroxine effect was absent when magnesium concentration reached 24 micro moles per millilitre Mudd *et al* (1955) demonstrated the antagonistic action of magnesium to thyroxine in its uncoupling effect the effect was also noted when triiodothyronine and triiodothyroacetic acid were the uncoupling agents

Conclusion

In spite of all the work which has been done on these *in vitro* effects the subject is far from clear The two main objections to the uncoupling effect being the major action of the thyroid hormones are (1) the very large concentrations of the hormones necessary to produce an effect and (2) its non specificity 2 4 Dinitro phenol which has been shown (Dodds and Robertson 1933) to bring no alleviation of symptoms in myxoedema apart from raising the basal metabolic rate is a very powerful uncoupling agent as is the antithyroxine agent *n* butyl 3 5 diiodo 4-hydroxybenzoate (Sheahan *et al* 1951)

Undoubtedly thyroid hormone effects at a subcellular level had to be explored but the results have been disappointing a physiological system rather than a biochemical one may well yield more fruitful results The dependence of the thyroid gland on the pituitary the adrenals and the pancreas indicates that other factors must be present for the thyroid hormone effect to be fully manifested

OTHER THYROID HORMONE LIKE COMPOUNDS

Besides thyroxine and triiodothyronine other iodinated diphenyl ethers have been demonstrated in the thyroid blood and tissues Roche and his co workers have detected 3 3 diiodothyronine and 3 3 5 triiodothyronine in small amounts in tissues of animals after ^{131}I injections the latter compound is almost inactive but the former is claimed to possess considerable activity 70 per cent and 82 per cent of the activity of thyroxine when studied by the amphibian metamorphosis test and by the goitre prevention method (Roche *et al* 1955a 1955b 1956b 1956c) Gemmill (1956) however has found only a low activity for this compound in stimulating oxygen consumption in thyroidectomized rats and in the goitre prevention assay

The keto acid analogues of thyroxine and triiodothyronine have been mentioned previously as metabolites of the thyroid hormones Roche *et al* (1955c 1956a) find potencies of tetraiodothyropyruvic acid to be 30 per cent of that of thyroxine in amphibia and in preventing goitre formation Comparable potencies for triiodo thyropyruvic acid were 100 per cent in both tests Pitt Rivers (unpublished) has obtained lower potencies for both compounds by the goitre prevention method 13 per cent for the tetraiodo acid and 20 per cent for the triiodo acid

The acetic acid analogues of thyroxine and triiodothyronine (Harington and Pitt Rivers 1952 Pitt Rivers 1953) have been variously assayed Triiodothyro acetic acid (triac) has considerable antigoitrogenic activity it can stimulate growth in thyroidectomized rats (Pitt Rivers unpublished) and can restore the plumage of thyroidectomized birds to a prethyroidectomy condition (Bruce *et al* 1954) The effects of triac in myxoedema and in normal subjects have been investigated by Lerman and Pitt Rivers (1955) and by Trotter (1955, 1956) It appears that triac may have a more marked effect on cholesterol and water meta bolism than on the basal metabolic rate this differential action is not shown with tetraiodothyroacetic acid (Goolden 1956)

The metabolism of triac has been studied by Roche *et al* (1956a) who found that in rats it is similar to that of triiodothyronine though its concentration by liver is somewhat lower and longer lasting Preliminary studies by Goolden and Pitt Rivers (unpublished) in man show an initial rapid disappearance from the blood followed by a much slower clearance

Triiodothyroacetic acid —This acid has been claimed (Thibault and Pitt Rivers 1955) to exert an immediate stimulation on respiration of tissue slices *in vitro* this finding has not been confirmed Heimberg *et al* (1955) also found an immediate effect with this analogue on glycolysis in ascites tumour cells *in vitro* No effect was detected with thyroxine and triiodothyronine It has been suggested that many tissues are relatively impermeable to the thyroid hormones in *in vitro* systems and lose their viability before they can exert their effects In this connexion Eaton *et al* (1953) failed to detect any immediate effect of thyroxine on influenza virus although dinitrophenol and *n* butyl 3 5-diiodo-4 hydroxybenzoate inhibited the growth of the virus in tissue culture Eaton *et al* (1956) found however that both thyroxine and triiodothyronine stimulate oxygen consumption in viruses in tissue culture after a latent period of 12 hours

A different manifestation of thyroid activity has been studied by Lachaze and Thibault (1951) and Harington and Thibault (1953) Thyroxine and triiodothyronine have both been shown to enhance the sensitivity of isolated rabbit intestine to adre naline but the effect only appears after a latent period With the amines thyroxamine and triiodothyronamine the effects are immediate and obtainable with very minute doses Lachaze and Thibault (1952) have presented chromatographic evidence of the presence of thyroxamine in the intestinal mucosa but this finding is still not established Not enough is known of the analogues described in this section to warrant their inclusion in a scheme of thyroid hormone metabolism

CONCLUSIONS

The following summing up of iodine metabolism in the body can be made according to our present knowledge

(1) Iodide is concentrated from the circulation by the thyroid gland

(2) The thyroid enzymes convert inorganic iodide to the organically bound

iodine in thyroglobulin The principal iodo amino acids in thyroglobulin are diiodotyrosine and thyroxine Monoiodotyrosine and 3 5 3 triiodothyronine are also present Recent evidence has been put forward for the presence of 3 3 diiodothyronine and 3 3 5 triiodothyronine in the thyroid

(3) The proteolytic enzyme of the thyroid liberates the iodo amino acids from protein linkage Monoiodotyrosine and diiodotyrosine are dehalogenated in the thyroid and they do not normally pass out of the gland The iodide thus liberated is presumably re utilized for thyroglobulin biosynthesis Thyroxine and triiodo thyronine pass into the circulation

(4) The principal circulating hormone is thyroxine Triiodothyronine is also present in the blood but only in small amounts

(5) The thyroid hormones are chiefly metabolized in the liver and kidneys In the liver thyroxine and triiodothyronine are conjugated as glucuronides and then pass via the bile into the gastro intestinal tract There is evidence of an alter native metabolic pathway in the liver oxidative deamination of thyroxine and triiodothyronine to give the corresponding pyruvic acid derivatives

(6) In man thyroid hormonal iodine is mainly excreted by the kidney as iodide in the rat much of the hormonal iodine is excreted in the faeces probably as thyroxine

(7) The enzymes in the thyroid which are responsible for the concentration of iodide from the blood and for its organic incorporation are unknown Equally unknown is the peripheral target of the thyroid hormones research at isolated enzyme levels has failed to reveal what might be a specific thyroid effect The dependence of thyroid function on other endocrine systems in the body indicates that the search for knowledge of the thyroid s action may demand a more physio logically integrated system than has yet been investigated

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CHAPTER 2

ANTITHYROID DRUGS

D M DUNLOP

ANTITHYROID agents are chemical substances which prevent the synthesis release or peripheral action of thyroxine The three main types of these compounds are ions of iodine the radioactive isotopes of iodine and the goitrogenic drugs The first and third of these compounds are discussed here radioactive iodine being dealt with in Chapters 4 and 5

IODIDE

Pharmacological action

Iodide is the oldest of the antithyroid drugs and until 1943 it was the only one known to have a specific effect in inhibiting the signs and symptoms of hyper thyroidism It is somewhat paradoxical that a drug which in very small quantities is necessary to prevent endemic cretinism should in larger doses have the effect of reducing the hypertrophy and hyperplasia of the hyperactive thyroid gland and of causing it to store thyroxine-containing colloid thereby greatly reducing the excessive amount of thyroid hormone entering the circulation How iodide does this is still largely unknown It is certain that its action is entirely different to that of the goitrogens and that it is without effect on the peripheral action of thyroxine since it has no influence on the hyperthyroidism produced by large doses of thyroxine or thyroid extract It is tempting to postulate that iodide produces its effect by inhibiting thyrotrophin (Astwood 1949) which has an exactly opposite effect to the involution of the thyroid and the storage of colloid in the gland—the characteristic results of iodide medication An inhibiting action of iodide on thyrotrophin would also explain its relatively poor therapeutic effect on a hyper functioning adenoma of the thyroid in comparison with its effect in Graves disease since an adenoma of the thyroid may be independent of the secretion of thyrotrophin Nevertheless since this hypothesis does not explain why the administration of iodide has no effect on normal thyroid function we are forced to confess our relative ignorance of its pharmacological action in hyperthyroidism (Rawson *et al* 1945)

Clinical effects

The therapeutic action of iodide on a hyperthyroid patient who has not previously had the drug administered is exceedingly dramatic and some effect may be noted within 24 hours The full effect of the drug is usually felt within 10–14 days During this time there is a marked fall in the basal metabolic rate a decrease in the intensity of all the symptoms with a gain in weight and a slowing of the pulse rate The gland itself becomes firmer and less vascular Complete euthy roidism is however seldom attained by this form of treatment alone

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of cabbage leaves tended to develop goitres an observation confirmed by others and extended to include brassica seeds and soya beans as goitre producing sub stances The changes in the thyroid were those of a diffuse parenchymatous goitre involving hyperplasia and loss of colloid

These earlier accounts of goitrogenic substances differed as to whether the changes produced could be inhibited by iodide as to the resulting metabolic state of the animal and as to the nature of the effective mechanism but they were the forerunners of the intensive studies which were begun by Griesbach Kennedy and Purves (1941) who found that the feeding of brassica seeds not only produced goitres but that simultaneous changes occurred in the pituitary similar to those which follow thyroidectomy Further it was discovered that the hyperplasia of the thyroid did not take place after hypophysectomy showing that the goitre was mediated by the anterior pituitary

In attempting a year later to find the causative factor in the rape seeds Kennedy (1942) suggested that it might be a derivative of thiourea and indeed showed that allyl thiourea caused the characteristic changes already noted in the thyroid and pituitary His observation was slightly anticipated by Richter and Clisby (1941) and by the Mackenzies and McCollum (1941) who showed respectively that thyroid hyperplasia occurred on administering phenylthiourea or sulphaguanidine an effect not influenced by adding iodide to the diet but which could be abolished by effective doses of thyroxine

Subsequent studies by the Mackenzies (1943) and by Astwood Sullivan Bissell and Jyslowitz (1943) showed that the hyperplasia of the acinar cells of the thyroid and the decrease in colloid of the follicles which followed the administration of thiourea were associated with a fall in the basal metabolic rate along with a decrease in growth and a diminished food intake The apparently paradoxical finding was thus made that a hyperplastic thyroid could be produced similar histologically to a thyrotoxic gland but associated not with hyperthyroidism but with hypothy roidism The classical work of Marine (1935) however had shown that the picture of thyroid hyperplasia and lack of colloid does not necessarily indicate thyrotoxicosis It may mean that the thyroid is producing insufficient hormone for the needs of the body and is being stimulated to hyperplasia by thyrotrophin from the pituitary to correct the deficiency Such hyperplastic glands are often seen when there is an increased hormonal demand as at puberty and pregnancy and in people taking iodine deficient diets When iodide is given under such circumstances the thyroid reverts to its normal state The giving of iodide how ever had no effect on the hyperplastic glands produced by thiourea showing that the effect was due to a different action

Pharmacological action

The clinical association of thyroid hyperplasia and hypothyroidism might have been explained on the basis that the goitrogenic agents neutralized thyroxine in the body tissues the thyroid being stimulated to hyperplasia by thyrotrophin in an attempt to correct the deficiency The fact that the administration of thyroxine entirely nullified the effect of the goitrogens made this explanation in turn inconclusive

These results suggested that the goitrogenic agents acted directly on the thyroid

Differences of opinion over prolonged iodide therapy

Opinions differ as to the course of the disease when iodide therapy is prolonged There is no doubt that after a few weeks it is usual for the pulse and metabolic rate to rise again for the symptoms to become aggravated and for a general deterioration to occur in the patient s health Many authorities consider that these manifestations indicate that the patient has become entirely refractory to iodide They believe that pharmacologically the drug has an initial valuable *effect which cannot be sustained and that this should be sedulously preserved for* the crucial pre operative period it should never be allowed to waste itself on the patient s ordinary existence Others however believe that patients do not really become iodide resistant but are still under the control of iodide in spite of a deterioration in their condition hyperthyroidism is known to pursue a cyclic course of remission and exacerbation and iodide is considered by some to hold these fluctuations in check at a level somewhat below that which would occur in the untreated case (Means 1948)

Opinion is largely unanimous however that iodide should seldom be prescribed in hyperthyroidism except for the 10–14 days prior to thyroidectomy (Plummer 1923) It is absolutely contra indicated if the patient is to receive radioactive iodine treatment in the near future and its immediate previous use may even interfere to some extent with the therapeutic action of goitrogenic drugs

Dosage

Iodide continues to be widely prescribed as Lugol s solution which contains a *mixture of 5 per cent iodine and 10 per cent potassium iodide in water* There seems no reason to prescribe iodide in this form since identical results are obtained by giving potassium iodide itself A maximum therapeutic effect in hyperthyroidism is obtained by giving as little as 6 milligrams of potassium iodide daily This quantity is contained in about 0 06 millilitre (1 minim) of Lugol s solution Most physicians prescribe larger amounts—1 grain of potassium iodide or 10 minims of Lugol s solution once or twice a day Although these larger doses do no harm they should never be exceeded It is wise to continue iodide medication for a week following thyroidectomy

GOITROGENIC (ANTITHYROID) DRUGS

Goitrogens are drugs which interfere with one or other of the mechanisms whereby thyroxine and triiodothyronine are synthesized by the thyroid The resulting hypothyroid state of the individual stimulates the anterior pituitary to produce an excess of thyrotrophin which results in thyroid hyperplasia During the last few *decades a large number of such agents have been described The effect of some* of these substances is due to the alterations in iodine metabolism which they induce since the simultaneous administration of iodine inhibits their goitrogenic action Certain other substances such as cyanide produce goitres presumably by inhibiting the biological oxidation of body cells

History and pharmacological action

Chesney Clawson and Webster (1928) first observed that rabbits fed on a diet

however several other compounds have been introduced and widely used The most important of these have been Methimazole (*U S P*) (Tapazole) and Carbimazole (*B P*) (Neo mercazole) which closely resemble each other chemically and in their pharmacological action Iothiouracil (Itrumil) differs from the other goitrogenic drugs in that an iodine atom is incorporated in the molecule in the hope that the compound would combine the antithyroid actions of both goitrogens and iodide Its effects however are much more like those produced by iodide than by thiouracil and iothiouracil is not generally regarded as a very desirable preparation

These drugs are rapidly absorbed from the gastro intestinal tract and are distributed throughout all the tissues and body fluids appearing in highest concentration in the bone marrow and thyroid They pass through the placenta easily and are secreted in the milk Their concentration in the blood 8 hours after administration is low though methimazole and carbimazole have a longer action Approximately 50 per cent of thiouracil is broken down in the body and 25 per cent is excreted in the urine but none in the stools Propylthiouracil is broken down less rapidly and high concentrations accumulate in the thyroid if 60 per cent did not become conjugated in an inactive form it might have an even more powerful antithyroid effect

Since the pharmacological actions of these drugs are very similar the choice of preparation depends upon the incidence of the undesirable side effects which they may produce and to a less extent upon their duration of action Although their potency varies greatly this should not alone be taken into consideration provided the dose required to produce a satisfactory inhibition of the synthesis of thyroxine is not such as to produce toxic effects Propylthiouracil methimazole and carbimazole in therapeutic doses are approximately equal in the frequency with which they produce toxic side effects and are less toxic than methylthiouracil Methimazole and carbimazole are less transient in their action than propylthiouracil and as carbimazole is freely available in Great Britain it is in the author s opinion the drug of choice

Potassium perchlorate

Certain anions particularly potassium perchlorate also possess a goitrogenic action (Wyngaarden Stanbury and Rapp 1953) and have been used with some success in the treatment of hyperthyroidism Their influence can be readily overcome by the administration of iodine a fact which implies that they interfere with the uptake of inorganic iodide by the thyroid though how they do this is as yet unknown The action of potassium perchlorate is slower than that of carbimazole and the thiouracils In addition they may produce gastric irritation so that on the whole they are less satisfactory drugs

Therapeutic applications

There are three main therapeutic applications of the goitrogenic drugs (1) the medical treatment of hyperthyroidism (2) the pre operative preparation of the hyperthyroid patient for thyroidectomy (3) the production of a myxoedematous state as an ancillary measure in the treatment of disease other than that of the thyroid

thus preventing the production of thyroxine and this was proved to be the case by Franklin Chaikoff and Lerner (1944) who studied the effects of goitrogens on the *in vitro* conversion of radioactive inorganic iodide to thyroxine and diiodo tyrosine by surviving thyroid slices They found that thiourea and its derivatives strongly depressed this conversion It is therefore practically certain that the action of such substances is to interfere with the synthesis of thyroxine by preventing the iodination of tyrosine Our present knowledge derived from the foregoing experimental work may be summarized as follows goitrogenic substance prevention of iodination of tyrosine lack of thyroxine compensatory increased production of thyrotrophin thyroid hyperplasia

The precise mechanism whereby these agents prevent the iodination of tyrosine remains in doubt Since their potency seems to correspond with their strength as reducing agents it would seem logical to search for a mode of action dependent on their reducing activity Astwood (1949) has suggested three such possibilities (1) they might compete with iodide as a substitute for the iodide oxidizing enzyme (2) they might reduce peroxide and thus prevent it from serving as a substitute for peroxidase (3) they might reduce any free iodine liberated before it had a chance to iodinate tyrosyl groups None of these possibilities has direct experimental support Nevertheless the third is very probable since these substances in the *thiol form are known to reduce free iodine with great rapidity in vitro*

Preparations

Astwood (1943) was the first to prove the clinical value of thiourea in the treatment of hyperthyroidism since when its derivatives have been extensively used therapeutically Principally as the result of Astwood s work it is now recognized that there are hundreds of substances which possess antithyroid goitrogenic properties to a greater or lesser extent Many of these are to be found among common foodstuffs including the turnip but it is improbable because of their weak action that these foods are responsible for any significant proportion of the cases of goitre and myxoedema commonly encountered

Two principal groups of drugs have been discovered to possess antithyroid properties (Pitt Rivers 1950) The first and most active group consists of thiourea and its derivatives The second group contains an amino benzene ring in its structure and includes the sulpha drugs and substances such as *para* aminobenzoic acid and *para* aminosalicylic acid The drugs in this latter group have not been used therapeutically for their antithyroid effects but are of importance since long continued use of drugs of this type for other purposes may have the undesirable side effect of causing goitre and hypothyroidism

Propylthiouracil methylthiouracil methimazole and carbimazole

Thiourea because of its unpleasant taste was soon abandoned as a therapeutic agent in favour of thiouracil which was practically the only goitrogenic drug used until the end of World War II Methylthiouracil was introduced in Denmark in 1944 as a cheaper and more easily prepared substance and for many years was the principal antithyroid drug used in Great Britain while propylthiouracil was more extensively favoured in the United States of America During the past few years

A gain in weight is a characteristic result of treatment and is often striking Weight which should be regularly recorded is one of the most reliable prognostic features in hyperthyroidism

Tachycardia

When a severe degree of tachycardia is present this feature may persist long after the other symptoms and signs have disappeared and it is often the last to remit under treatment Thyrotoxic fibrillation returns to normal rhythm spontaneously in about 50 per cent of cases when a euthyroid state has been induced by the drugs just as normal rhythm may occur spontaneously after thyroidectomy When fibrillation persists quinidine treatment after full digitalization may restore the rhythm to normal

Glycosuria

The mild glycosuria so commonly encountered in thyrotoxicosis may disappear under the influence of antithyroid drugs just as it does after thyroidectomy There are of course thyrotoxic cases associated with true and severe diabetes the latter may be ameliorated but not cured by such treatment For such cases dietetic and insulin treatment will be required

Exophthalmos

Although exophthalmos may not be greatly benefited by drug treatment some improvement usually occurs in those patients who are going to experience a permanent remission as the result of treatment Occasionally it gets worse as it sometimes does after thyroidectomy This drug treatment however is less likely to give rise to the distressing condition of exophthalmic ophthalmoplegia than thyroidectomy

The drugs invariably cause the goitre to become softer and more vascular in contrast to the firmer and less vascular gland produced by iodide and the incidence of a permanent remission is much higher in those patients who show a significant decrease in the size of the gland as the result of treatment An increase in its size associated with symptoms and signs of hypothyroidism occurs as the result of over dosage as under such circumstances the pituitary is stimulated to produce an excess of thyrotrophin This hyperplasia and hypothyroidism disappears when treatment is stopped or the dose appropriately reduced but unless care is taken very large and unsightly goitres which may take a long time to recede can be produced by over dosage

Toxic reactions

The recorded signs of toxicity to these drugs (van Winkle *et al* 1946) include in order of severity slight oedema of the legs and feet conjunctivitis adenopathy rashes acute sensitivity reactions such as drug fever and vomiting and blood dyscrasias particularly agranulocytosis Eighty per cent of such reactions occur within the first 2 months of treatment especially when the patient is receiving the initial high dosage or when treatment is reinstituted after an interval of time Serious signs calling for a cessation of treatment are the acute sensitivity reactions and the blood dyscrasias In the treatment of out patients frequent white blood counts are impracticable as a means of guarding against agranulocytosis which in any case may be very sudden in its onset Patients should therefore be warned to stop the drug and report to the doctor should any untoward symptoms arise

THE MEDICAL TREATMENT OF HYPERTHYROIDISM

Dosage

The antithyroid drugs are given by the mouth in tablet form The usual dose of propylthiouracil or methylthiouracil for the first few weeks of treatment varies from 0 3 to 0 6 gramme a day depending on the severity of the disease As these drugs are rapidly excreted they should during this initial period be given in divided doses 3 times a day Carbimazole is roughly 10 times as potent as the thiouracil preparations so the equivalent range of dosage will be 30–60 milligrams a day Once a significant remission has been produced by this initial treatment the dose should be reduced to a maintenance level since a continuation of the initial high dosage would result in myxoedema and the indirect effect of excess secretion of thyrotrophin which would cause enlargement of the goitre The time required to produce an approximately euthyroid state varies from patient to patient The response is not so rapid as with iodide but is more consistent is progressive and can be maintained it usually takes from 3 to 5 weeks Patients with large nodular goitres are apt to be somewhat resistant to this form of treatment In such cases the initial large doses may have to be maintained for a longer period

The optimum maintenance dose also varies from patient to patient It is usual to administer 0 2 gramme of propylthiouracil or 20 milligrams of carbimazole daily to begin with reducing this according to the reaction of the patient to 0 1 gramme or 10 milligrams respectively and finally to 50 milligrams or 5 milligrams In mild cases 0 1 gramme and 10 milligrams may be given as the initial maintenance dose A return of thyrotoxic signs and symptoms or an enlargement of the goitre associated with symptoms of hypothyroidism indicates the need for an appropriate increase or decrease of the maintenance dose Treatment has to be continued for at least a year and sometimes longer

Clinical effects

As the store of thyroxine in hyperthyroid glands is very small an effect is rapidly produced in hyperthyroidism if further formation of the hormone is prevented Some subjective benefit is usually experienced within a week of starting treatment thereafter symptoms steadily improve The objective signs parallel in their improvement the subjective sensations of the patient

After a latent period of about a week a fall in the basal metabolic rate occurs and with optimal doses it may become approximately normal in the majority of cases in from 3 to 5 weeks when the change should be made from initial to maintenance dose The determination of the basal metabolic rate however may not be a practicable possibility in ordinary practice and it certainly should not be attempted in an out patient Even in hospital patients become over anxious about such tests and artificially high figures may in consequence be obtained A far better guide to dosage is to rely on clinical observations—the feel of the hand the state of the pulse the weight and the subjective sensations of the patient

The blood cholesterol concentration is low in untreated hyperthyroidism just as it is high in myxoedema and it tends to rise under the influence of antithyroid drugs It is not however a reliable yardstick for the control of treatment as in individual cases there is often little correlation between blood cholesterol concentration and the progress of the patient

Treatment for young people

Antithyroid drugs constitute the best treatment for most young people especially those suffering from moderate primary Graves disease and unobtrusive goitres The disease in such cases is usually self limiting and it is only necessary to maintain a euthyroid state till spontaneous remission occurs The idea of irrevocable surgical interference with the endocrine system of young patients is repugnant and if possible should be avoided it is always possible to resort to thyroidectomy if they relapse after treatment or if serious toxic reactions to the drugs occur

In the author s opinion these drugs also constitute a satisfactory form of treat ment for hyperthyroidism in the pregnant woman Surgery is undesirable in late pregnancy and radioactive iodine is absolutely contra indicated The drugs however are secreted in the milk the patient should not therefore be allowed to nurse her baby The danger that the function of the foetal thyroid may be depressed by the drugs and that owing to their goitrogenic action the child may be born with a goitre is not serious provided care is taken to avoid over dosage with the production of hypothyroidism in the mother (Astwood 1951)

Treatment for patients over 35 years of age

Thyroidectomy is indicated for most patients between 35 and 45 years of age especially for those with definitely nodular goitres with large and unsightly goitres with goitres causing pressure symptoms and with goitres which are retrosternal or with retrosternal extensions In the future radioactive iodine may prove to be the treatment of choice for the majority of cases of thyrotoxicosis At present it should be reserved for the treatment of patients over 45 years of age or for those who develop a recurrent thyrotoxicosis following thyroidectomy There is a considerable risk of a second operation in such cases causing hypoparathyroidism or vocal cord paralysis

Though these are generalizations to help the practitioner in his choice of treat ment it is best not to adhere to rigid rules but rather to treat each patient as an individual problem The type of therapy selected should take the widely varying individual circumstances of each patient into account Medical treatment by drugs has the advantages of their general availability their comparative safety and the fact that mild cases can be treated as out patients without the necessity in many cases of the patient stopping work Their main disadvantages even in suitable cases are the long period of treatment and observation required without any certainty of a final permanent remission and the need for intelligent co opera tion on the part of the patient during treatment

Pre-operative use of antithyroid drugs

Opinion is largely unanimous that these drugs have an important role to play in the preparation of thyrotoxic patients for operation Iodide medication alone seldom restores the metabolism of a severe case to normal It greatly improves the symptoms and signs but does not hold them in complete check as is usually the case when adequate treatment is given with antithyroid drugs The pre operative use of the latter has greatly diminished the incidence of post operative thyrotoxic crises and has thus still further reduced operative mortality The thyroid glands of patients treated in this way are rendered excessively vascular and this

and particularly on the first sign of a sore throat Provided they do this at once serious toxic reactions are not likely to make this treatment unsuitable for use in general practice and when the thyrotoxicosis is only mild the patients may be successfully treated from the outset as out patients In the vast majority of cases when the administration of the drug is stopped and appropriate antibiotic therapy instituted the agranulocytosis soon disappears Most of the recorded deaths from agranulocytosis due to these drugs occurred between 1943 and 1945 when antibiotics were not as freely available as they are now Even then the mortality rate compared extremely favourably with that of expert surgery

Toxic manifestations were rather frequent when the older antithyroid drugs were used About 11 per cent of all patients treated showed a greater or less degree of intolerance and in about 7 per cent the toxic effects were sufficiently serious to necessitate the abandonment of this form of treatment Agranulocytosis occurred in from 1 to 2 per cent of cases The incidence of toxic reactions resulting from propylthiouracil or carbimazole is much less In a fairly extensive experience of the use of the latter drug the author has not encountered a single toxic reaction in a patient taking less than 20 milligrams of carbimazole a day

Mild signs of intolerance to one preparation can be avoided by the use of an alternative drug but once a serious reaction has developed to a particular drug it is usually unwise to continue treatment with any similar compound

Ultimate effects

In hyperthyroidism treatment with antithyroid drugs should be continued uninterruptedly for at least a year There is much evidence to suggest that a high relapse rate follows shorter periods of treatment Among patients who have been treated adequately a permanent remission may be anticipated in about 50 per cent of all cases of hyperthyroidism In mild cases of primary Graves disease in young women with unobtrusive goitres the proportion of cases permanently cured by this method of treatment approaches 70 per cent whereas in middle aged women with nodular goitres the proportion of permanent remissions is not more than 40 per cent The likelihood of a permanent remission is increased if it has been possible to maintain the patient in a euthyroid state for some months by as small a daily dose as 50 milligrams of propylthiouracil or 5 milligrams of carbimazole and if the goitre has decreased in size and the exophthalmos receded The majority of relapses take place within 2–4 months of stopping treatment and if the patient remains well for 6 months relapse is unlikely to occur Those who relapse once after adequate treatment can of course be treated again but the majority will relapse once more after a second course so that for them alternative methods of treatment are usually indicated (Dunlop and Rolland 1950)

Indications and contra indications in antithyroid drug treatment

There is a considerable difference of viewpoint as to the place of these drugs in the therapeutics of hyperthyroidism Some authorities believe that they should seldom be used except to control severely thyrotoxic patients prior to pre operative treatment with iodide or prior to the administration of radioactive iodine Others believe that they are the treatment of choice in the majority of patients and that alternative methods should only be resorted to if they fail Between these two extreme views the following generalizations are acceptable to many workers

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causes technical difficulties for the surgeon at operation The ideal pre operative treatment is the use of both drugs The patient is rendered euthyroid by antithyroid drug treatment its administration is then stopped and iodide given for the 10-14 days immediately preceding the operation with the result that the gland becomes firmer and less vascular Care should be taken not to make the patient hypothyroid since a myxoedematous patient is not a good operative risk (Cole and Fowler 1948) being peculiarly sensitive to morphine and anaesthetics

Production of therapeutic myxoedema

In the past myxoedema produced by total thyroidectomy has sometimes been advocated for the treatment of intractable angina of effort and heart failure The rationale is to lessen the discrepancy between the inadequate circulation and the oxygen demands of the patient Furthermore it is claimed that in the absence of thyroxine the myocardium is less sensitive to sympathetic nervous stimulation The major operation of total thyroidectomy however carries with it a considerable risk in such patients and the induction of myxoedema by antithyroid medication would seem to be a preferable alternative It must be remembered that it takes some 3 months to produce myxoedema by such methods since the euthyroid in contradistinction to the hyperthyroid gland has a large store of thyroxine containing colloid in its follicles This prevents the patient from becoming myxoedematous for a time even though no new thyroid hormone is being synthesized (Waitzkin 1951) Retreat in fact is always possible by stopping the administration of the antithyroid drug but treatment with radioactive iodine would appear to be a better and quicker way of obtaining the same result (Blumgart and Freedberg 1952) This therapeutic induction of myxoedema in heart disease has a very limited application It occasionally prolongs life but only by exchanging one form of misery for another which may not be quite so poignant

GOITRE AND MYXOEDEMA AS TOXIC SIDE EFFECTS OF OTHER DRUGS

PAS

A number of drugs containing an amino benzene ring in their structure though not used for their antithyroid effects are goitrogenic and when employed thera peutically for other purposes may have the undesirable side effect of producing goitre and hypothyroidism The most important of these is *para* aminosalicylic acid (PAS) Its use as an antituberculous remedy in heavy dosage and for long periods at a time not infrequently results in thyroid hyperplasia with myxoede matous symptoms and signs Indeed Macgregor and Somner (1954) have reported that of 83 tuberculous patients treated with 20 grammes daily of PAS for 5 months or more 20 developed a goitre often with associated signs of hypothyroidism Although the goitrous and hypothyroid state induced by PAS is usually quickly reversible when treatment is stopped it seems that occasionally irreversible and perhaps permanent degenerative changes may be produced in the thyroid by the long continued use of this drug In practice if clinical hypothyroidism and a goitre develop in a patient receiving PAS thyroxine or thyroid extract should be given and if PAS must still be used continued concurrently Alternatively isoniazid can be substituted for PAS Iodide has no beneficial effect on the goitres produced by PAS suggesting that the latter s goitrogenic action resembles that of

the thiouracil rather than the perchlorate group of drugs which is readily overcome by the administration of iodide

Resorcinol

Resorcinol may be absorbed through the skin or from ulcerated surfaces and it has been known for years that a hyperplastic vascular goitre with myxoedema may result from the prolonged application of resorcinol ointment to varicose ulcers On withdrawing the drug the myxoedema subsides and thyroid function returns to normal (Bull and Fraser 1950) The goitrogenic action of resorcinol is probably that of other phenol-containing compounds and resembles thiouracil rather than perchlorate

Thiocyanate

Thiocyanate which was used fairly extensively some years ago in the treatment of hypertension occasionally produced goitre and hypothyroidism (Barker Lindberg and Wald 1941) Its effect could be abolished by giving iodide The goitrogenic mechanism is similar to that of perchlorate though weaker and more capricious

Sulphonamides

Though sulphonamides in the experimental animal have been shown to have a goitrogenic action similar to that of thiouracil this effect has rarely been observed in man as such drugs are not used in large doses for more than a short period of time Radioactive iodine studies have demonstrated slight depression of thyroid function in patients undergoing such treatment and it has been reported that a myxoedematous diabetic patient receiving N butyl N sulphanilylurea (BZ 55) as an oral insulin substitute required in consequence a significant increase in thyroid dosage (Duncan Baird and Dunlop 1956)

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CHAPTER 3

THE AETIOLOGY AND TREATMENT OF EXOPHTHALMOS AND EXOPHTHALMIC OPHTHALMOPLEGIA

I C GILLILAND

IN A discussion of the many problems involved in exophthalmic ophthalmoplegia Brain (1952) remarked that there was an exception to any statement that could be made This remains true and makes it unlikely that any simple explanation can be found to fit all the facts known at present about this condition and the other eye signs which accompany Graves disease Nevertheless there has been con siderable progress towards a solution of the problem, and it is worth while re examining the theories of causation in the light of recent work

THE CLINICAL PROBLEM

Broadly speaking Graves disease has at least two major components—an over active thyroid gland and eye signs Both features are usually present in which case there is little diagnostic difficulty When eye features predominate Means (1945) has suggested the name hyperophthalmopathic Graves disease There are exceptional cases in which the eye features appear to be present alone and for these the term exophthalmic ophthalmoplegia (Brain and Turnbull 1938) seems most suitable In fact there is every grade of variation between these con ditions Most physicians take the view that they are basically the same and that the eye signs have a common aetiology whether thyrotoxicosis is present or not

Eye signs

The eye signs of Graves disease have in the past been graced with many epony mous descriptions but simplification has now led to their classification into four main categories (Fig 1)

Lid lag —By this is meant an apparent increase in tone especially of the levator palpebrae superioris and it gives rise to the staring appearance and jerky or spastic movements of the upper eyelid when the eye follows the examining finger up and down It is the commonest eye sign but one which may be present in a nervous person and so is of limited diagnostic importance in Graves disease Caution should be applied to the claim that this eye sign can be produced by feeding thyroxine to a normal person it is not usually a feature of thyroid addiction It differs from the other eye signs of Graves disease in that treatment of the thyrotoxic aspect usually removes it the tenseness relaxes and the lid lag improves This may improve the appearance of the patient and give rise to the false impression that the other eye signs are progressing favourably (Dobyns 1950)

Exophthalmos —This is the forward protrusion and proptosis of the eyeball which is almost invariably asymmetrical at first and may at this stage appear to be unilateral Sooner or later however both eyes are usually involved

Ophthalmoplegia —This is due to weakness of the external muscles which move the globe and the degree usually parallels the degree of forward protrusion of the eye though this is not always the case These features are not necessarily associated and cases are encountered with gross protrusion still capable of move



FIG 1 —*The eye signs of Graves disease note lid retraction especially on right side* exophthalmos especially on right side ophthalmoplegia causing evident strabismus on upward movement and oedema beginning at inner canthus

ment of the eyeball in all directions there are also cases with almost fixed eyeballs which hardly appear to protrude As the muscle weakness is also asymmetrical there is likely to be difficulty in keeping the eyes in the same axis on movement It is this muscular weakness which when severe leads to diplopia The muscles subserving upward and outward movement are usually those most affected and hence diplopia is most often noticed in those directions

Oedema —This is commonly associated with subjective symptoms of irritation pain and lachrymation and objective signs of worsening of the exophthalmos it can be an ominous eye sign A watery swelling of the scleral conjunctiva with a suffused appearance of its vessels commences in one or other canthus There may be considerable oedema of both lids The oedema of the conjunctiva may spread and tend to evert the lower lid interfering with normal tear duct drainage and increasing lachrymation The presence of oedema should be taken seriously because it may herald an *alteration in the character of the eye features They may* become a threat to sight and even to life the term malignant should be res tricted to this type of exophthalmos The distinction which is commonly accepted between essential and malignant hypertension affords a good parallel Malignant

exophthalmos is an uncommon event compared with the more ordinary forms and like malignant hypertension it may start without warning and pursue a quickly adverse course or the eye signs of a long-continued Graves disease may suddenly enter a malignant phase

PATHOLOGY

Many of the eye signs in particular the exophthalmos result from overfilling of the rigid orbital cavity Expansion of the orbital contents can only lead to pro trusion forwards as this is the only direction in which movement can occur (Rundle and Wilson 1944) For mechanical reasons this protrusion is most readily observed in the lateral portion of the upper lid above the medial canthus and as a lumpy bulging in the lateral portion of the lower lid

Increased bulk in retro-orbital tissues

The nature of the substance causing the increase in bulk of the retro orbital tissues is not yet completely decided it possibly varies with the stage and duration of the eye condition Necropsy studies (Rundle and Pochin 1951) in 17 cases many of which were of long standing and none of which had malignant exophthalmos showed that the increase in bulk in the orbital cavity in these cases was mainly due to increased fat in the orbital fibro fatty tissues also the average fat content of the eye muscles was doubled

Oedema of the connective tissues

On the other hand Naffziger (1954) is emphatic that the changes which he observed during decompression operations in the malignant phase of exophthalmos were those of oedema and in an earlier phase biopsy specimens (Smelser 1937) also showed oedema of the connective tissue these too involved the fat and muscles Along with this oedema there was an infiltration of wandering cells some of which were in aggregates resembling germinal centres The oedematous infiltrate was particularly noticeable in some muscles and not uniform even in the muscles of one orbit The suggestion has recently been made that there is an increase of mucopolysaccharides particularly responding to hyaluronidase in the increased orbital contents (Ludwig Boas and Soffer 1950 Asboe Hansen and Iversen 1951) An increase of this material which attracts water could possibly explain the oedema

Clearly the changes so far observed are disappointingly non specific being those of a generalized chronic inflammation in which oedema predominates in the acute or malignant phase whilst fatty infiltration and scarring is the subsequent and final outcome

AETIOLOGY

Exophthalmos and anterior pituitary extracts

Obviously a condition which is so variable in its clinical and so unspecific in its pathological manifestations will be the subject of much speculation Earlier theories of overactivity of the cervical sympathetic nerves causing the contraction of the smooth muscle of Muller (Code and Essex 1935) and of relaxation of the

extra ocular muscles are no longer held Present views centre around the anterior pituitary hormones and have their origin in the work of Marine and Rosen (1934) who found that anterior pituitary extracts could cause exophthalmos and goitre in rabbits and that the exophthalmos occurred more readily in the absence of the thyroid gland Smelser (1937) repeated their observations on guinea pigs and drew attention to the histological similarity of the retrobulbar tissues in the animal rendered exophthalmic with anterior pituitary extracts and in clinical Graves disease in man Many other observers have confirmed this experimental finding (Paulson 1937 Aird 1941 and Dobyns 1946) so that it is indisputable that such anterior pituitary extracts can cause exophthalmos in animals

Thyroid stimulating hormone

The convenient use of the term thyroid stimulating hormone (T S H) to mean an anterior pituitary extract rich at least in this substance has led to much confusion

It is unlikely that the anterior pituitary extracts used in the above experiments contained only one trophic factor and it is also unlikely that they were in a pure state The experimental data therefore only show that a substance causing exophthalmos in animals can be produced from such anterior pituitary extracts which are rich in thyroid stimulating hormone It cannot be concluded that T S H itself is the cause of the exophthalmos for it is possible that denatured or altered T S H *or some totally different substance extracted by the same procedure* may be responsible Dobyns (1946) observed that highly purified anterior pituitary extracts had very little effect in producing exophthalmos in animals but Jefferies (1949) by iodinating anterior pituitary extracts inactivated the principle producing thyroid stimulation but left intact a factor with some ability to produce exophthalmos Later Dobyns and Steelman (1953) were able by repeated extraction to produce two anterior pituitary residues the one which stimulated the thyroid without producing exophthalmos they called T S H the other which produced exophthalmos with a minimal stimulation of the thyroid they called exophthalmic producing substance or E P S Further investigation of the nature of the exophthalmos producing substance by Smelser and Ozanics (1954) in chicks showed that the ability of a pituitary extract to produce exophthalmos was not necessarily proportional to its ability to induce thyroid hyperplasia They confirmed that E P S although obviously closely associated was not identical with T S H

Measurement of T S H and E P S

The above experimental data in animals has been associated with attempts to measure T S H and recently E P S in clinical thyroid diseases Present methods of measuring these substances in blood or urine are necessarily difficult biological techniques and subject to the wide errors of such methods they differ from one another in so many respects that the data are not readily comparable However Gilliland and Strudwick (1956) have confirmed that a high level of T S H can occur in cretins or patients with induced myxoedema who do not have eye signs The corollary of severe eye signs without high levels of T S H has also been reported Such findings support the earlier contention (de Robertis 1948 Purves and Griesbach 1949) that T S H is not the cause of exophthalmos In fact both clinical and animal studies show that some factor other than T S H must be involved Dobyns and Wilson (1954) reported their attempts to assay E P S by their ingenious

Atlantic Minnow technique and their preliminary data appeared to show that E P S may circulate in measurable amounts However the delay in response in animals given test serum compared with the control animals given E P S control serum raises the question as to whether it is indeed the same substance which is being measured

Summary

It may be said that the eye signs of Graves disease are at present believed to be caused by a pituitary factor conveniently referred to as E P S but which is not identical with T S H though probably closely related to it Chemical analysis of the two substances derived by Dobyns from pituitary extracts would help greatly to elucidate this problem It seems difficult to concede that E P S can be a naturally occurring hormone but more likely that it is a degradation product produced *in vitro* by certain extraction procedures and in the anterior pituitary when it is deranged It could then be postulated that very occasionally a primary disorder of the pituitary leads to the production of E P S and so to the condition of exophthalmic ophthalmoplegia On the other hand in Graves disease more commonly both T S H and E P S are produced in varying amounts If such a supposition is correct the sudden removal of an overactive thyroid by surgical or medical means could lead to a sudden increase in activity of the pituitary and a greater risk of abnormal E P S secretion This is precisely the situation in which there would be the greatest risk of a malignant phase of exophthalmos arising

TREATMENT

Treatment of the established case is acknowledged to be extraordinarily difficult and it is accepted that the longer the eye signs have been present the less successful is it likely to be However even in long standing and inactive cases cosmetic surgery has much to offer in closing the palpebral gap or correcting alignments of the axes of the eyes

Thyroxine

In the light of our only partial knowledge of the aetiology the prime aim of treatment at present must be preventive Whether the thyrotoxic aspect of Graves disease is to be treated medically or surgically the objective is to prevent the abrupt alteration in pituitary state which this may entail by the simultaneous maintenance administration of thyroid hormones (Fraser and Wilkinson 1953) It has long been known that the simultaneous administration of exogenous thyroxine will prevent goitre formation during the administration of antithyroid drugs (Astwood *et al* 1943 Purves 1943) Dobyns (1945) showed that thyroidectomy leads to an increase in the prominence of the eyes in some cases and Dobyns and Haines (1946) indicated that their worsening with the onset of myxoedema could be reversed at least partly by desiccated thyroid Fraser and Wilkinson (1953) administered 0 3 milligram of thyroxine daily along with antithyroid drugs in the medical treatment of Graves disease Where eye signs were already severe 0 6 milligram daily was administered In none of 32 cases were the eye signs made significantly worse In fact the results of this combined treatment were as satisfactory in

controlling the thyrotoxic component of their Graves disease as in any other published series and the series is large enough to show that this combined method prevents the worsening of the signs

Cortisone

The oedematous inflamed appearance of the eyes in some cases has been reported to be improved by cortisone although the results in a planned Medical Research Council (1955) trial have been disappointing

Radiotherapy

Irradiation of the orbital cavity is considered to help selected cases with acute oedematous swelling (Jones 1951) Based on this work a convenient method is one designed to deliver 400–600 roentgen to the retro orbital tissues fractionated into 4 weekly treatments Each orbit has two fields of 6 × 4 centimetres one anteriorly and the other laterally The lens is protected by a lead disc set in a perspex jig which fits over the anterior aspect of the eye Successful attempts to irradiate the pituitary itself have been reported although it is known that the gland is peculiarly resistant to radiation It may be that during this procedure the orbital cavities receive a sufficiently adequate irradiation dose to account for the reported successes

Hyaluronidase

A recent development has been the introduction of hyaluronidase into the sub conjunctival space (Laurent and Scopes 1955) This would be rational if the main substance causing the increase of bulk were hyaluronic acid

Use of the eye signs as guides to treatment

It must be emphasized that whatever treatment is employed regular watch must be kept on the progress of the eye signs though exact means of doing this by the Hertel exophthalmometer to measure protrusion or by orbitonometer (Copper 1948) to measure orbital compressibility are not sufficiently refined to justify routine use Moreover the orbitonometer is not free from danger in compressing an orbit already at risk from corneal ulceration A useful indication of progress (Gilliland 1954) can be obtained by using a field of vision chart charting the range of movement of the ocular muscles and marking the distance to which the individual eye can move Regular checks of visual acuity are also essential as sudden deterioration of vision can be a most urgent sign of impending disaster

Surgical treatment

Should deterioration occur in spite of all available measures recourse must be had to surgical orbital decompression before the sight is lost Naffziger (1954) who has had a lifelong interest and wide experience recommends a large operational procedure Most surgeons still use this method although Rowbotham and Clarke (1956) suggest that a lesser procedure gives adequate results In the end serious threats to sight may have to be countered by liberating the eyeballs from their strangulating tension

Spontaneous regression

It is of some consolation to remember that the natural history of this disease is such that it may regress spontaneously at any stage and that very few cases require actual decompression whilst many improve whatever form of treatment has been adopted

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CHAPTER 4

TESTS OF THYROID FUNCTION

A G MACGREGOR and E J WAYNE

INTRODUCTION

THE DIAGNOSIS of thyrotoxicosis can usually be made on the history and physical examination alone but some patients do not present the classical features of the disease and the true nature of their complaint may be overlooked This is likely to occur when there is no obvious enlargement of the thyroid gland and when eye signs are absent The patients who present the greatest difficulty are those with a simple goitre or who after thyroidectomy have non specific features such as nervousness and tachycardia A survey by one of the authors (Wayne 1954) led to the conclusion that diagnostic decisions based on symptoms and signs alone were relatively accurate in the male but often unreliable in the female and later experience has confirmed this view Diagnosis based on clinical features may be extremely difficult in older patients with auricular fibrillation

Frank myxoedema once suspected is usually more easily confirmed on clinical grounds alone but lesser degrees of hypothyroidism as seen in hypopituitarism and in treated hyperthyroidism may be difficult to recognize Most clinicians would admit that confirmation of their clinical opinion is welcome in both hyper thyroid and hypothyroid states Objective tests of thyroid function usually play an essential part in the final diagnosis and are also of help in assessing the results of treatment

The available tests measure different aspects of thyroid function and will be dis cussed separately and subsequently an attempt will be made to assess their relative value

THE BASAL METABOLIC RATE

Estimation of the basal metabolic rate is still the standard laboratory investigation used by most clinicians when they wish to confirm a diagnosis of hyperthyroidism or hypothyroidism It has the advantage of being comparatively easy to carry out and gives some idea of the degree of severity of the disease It is a measure of increased or decreased metabolism

High metabolism

A raised B M R is found in a variety of other conditions which include (1) diseases of the blood such as leukaemia and polycythaemia (2) cardiovascular diseases for example heart failure and arteriovenous aneurysm (3) other endo crine disorders especially phaeochromocytoma acromegaly Cushing s syndrome and hyperparathyroidism (4) drug ingestion especially of thyroid extract and of

drugs with an amphetamine like action (5) psychoses and neuroses with agitation and organic nervous disease with involuntary movements such as paralysis agitans (6) fever and pregnancy A more complete list with references is given by Bauer (1956) In practice the presence of one of these conditions giving rise to hyper metabolism is usually obvious and B M R estimations are not carried out

Normal metabolism

A thyrotoxic patient may have a B M R which lies within the statistically acceptable normal range Skanse (1949) referred to numerous studies reporting the occurrence of normal B M R in 10–40 per cent of cases of hyperthyroidism and in our series 35 per cent had rates lying below + 20 per cent (Goodwin *et al* 1951) Further experience however has led to a modification of our views on the degree of inaccuracy of B M R estimations If the technique and standards advocated by Robertson and Reid (1952) are adopted much more consistent and reliable readings are obtained Duplicate readings are now carried out on at least two consecutive days and with the patient in hospital overnight and after the administration of full doses of butobarbitone Using this method a better clinical correlation is obtained although a less time-consuming method has been described by Fraser and Nordin (1955) It probably gives even more reliable results but involves a heavier degree of sedation

Low metabolism

A low B M R may be due to secondary hypothyroidism from hypopituitarism and a diagnosis of myxoedema should never be made on this test alone as a B M R below − 25 per cent (du Bois) occurs in simple obesity anorexia nervosa eunuchoidism and amenorrhoea (Barron 1956)

In practice much help can be obtained from accurate B M R estimations but when technique or standards are imperfect results can be most misleading

SERUM PROTEIN BOUND IODINE

The estimation of the amount of circulating iodine (^{127}I) bound to protein is generally regarded as an index of the amount of circulating thyroid hormone Several groups of workers in the United States of America have produced evidence that levels outside the normal range are the most reliable laboratory evidence of abnormal thyroid function The subject is well reviewed by Rapport and Curtis (1950) Starr *et al* (1950) think that the determination of the serum protein bound iodine is a reliable routine diagnostic measure and that it is of particular value in mild myxoedema and in iodine deficiency The normal range is from 3 5 to 7 0 micrograms per 100 millilitres (Fraser 1956) The level is raised in severe thyro toxicosis but in mild cases may be within the normal range It is also raised after the administration of both inorganic and organic iodine compounds

Other observers however have found the test less satisfactory In Great Britain De Mowbray and Tickner (1952) stated that the estimation was inadequate in itself as a test of thyroid function and Bauer (1956) made the comment that most clinics are not able to solve the problem of contamination and have abandoned this procedure as a routine test of thyroid function

The writers have both encountered serious technical difficulties with three different methods

RADIOACTIVE IODINE

The tests already discussed measure one aspect of thyroid function the protein bound iodine in the serum being an expression of the amount of circulating hormone derived from the gland and the B M R measuring the effect of the hormone on the oxygen consumption of the peripheral tissues

Measuring iodine metabolism

Radioactive iodine makes it possible to measure the dynamic aspects of iodine metabolism The isotope is used to label the iodide pool in the body and its subsequent distribution can be followed The thyroid gland collects iodide from the circulation most of the balance being excreted by the kidney The iodide is then incorporated into the molecule of thyroxine which is released back into the circulation These various steps can be measured with one of the isotopes of iodine that commonly used being ^{131}I although ^{13} I has useful applications as well

Physical qualities

^{131}I has many of the ideal physical qualities for such investigations its rate of decay is convenient its half life being 8 days This is not so short as to make undue haste necessary in its use nor so long that excessive radiation is delivered to the tissues The energy released by its decay has characteristics which make it easy to detect and relatively safe to use Furthermore the isotope can now be easily and cheaply obtained in what is virtually a state free from radioactive iodide so that no pharmacological effect of iodine is produced

Instruments and equipment required

Electronic equipment is necessary for the performance of radioactive iodine tests and the type used will depend on the scope of the investigations Urine samples can be conveniently counted by a Geiger Muller liquid counter for ease of working it is sufficient to count urine samples made up to a known volume in Winchester bottles the whole urine sample in the bottle being placed within a ring of counters (Veall and Vetter 1952) Blood samples are best analysed in a well or annular type of scintillation counter

In vivo counting techniques

In vivo counting techniques depend for their accuracy on a number of factors Allowance has to be made for varying gland size and counts due to backscatter and extrathyroid radioactivity must be eliminated Freedberg *et al* (1950) devised a simple and accurate technique which involves the examination of the patient within a ring of four scintillation counters placed equidistant in a circle with a radius of about 40 centimetres from the neck of the patient Alternatively counts may be recorded at a distance of 20–30 centimetres from the neck by a single scintillation or Geiger counter with suitable shielding arrangements so that true thyroid counts are obtained

Dosage

The dose of radio-iodine to be employed is of importance Measurements on urine samples alone need no more than 5 microcuries accurate gland measurements about 10

microcuries or more and blood studies even with sensitive equipment 25 microcurie doses Even with quite large tracer doses the radiation dose to which the whole body is subjected is under one rad although a 10-microcurie dose delivers approximately 15 rads to the thyroid gland There are of course wide variations in such gland doses which depend on the level of thyroid uptake and the rate of elimination of the isotope from the gland

Types of test

Thyroid uptake tests

When the thyroid gland is in a steady state the rate at which it secretes iodine bound to hormone into the circulation should be equivalent to the rate at which iodide is concentrated by the gland

Measurements of gland uptake are a commonly accepted parameter of gland function and theoretically the maximum uptake achieved should be recorded The time at which this occurs however obviously varies To avoid a long series of measurements an arbitrary time is chosen which is not so late that appreciable discharge from the peak level has occurred nor so early that there has been little thyroid accumulation with extrathyroidal tissue concentrations high

Uptakes after 24 hours were at first a popular index and widely used as screening tests of thyroid function It is not easy however to compare values from different groups of workers and the American ranges for normal uptakes (Werner 1955 McConahey *et al* 1956) are lower than those reported by British observers (Goodwin *et al* 1951 Fryers 1956) This is probably related to the varying stable iodine intakes in the different countries Values over 55 per cent of the administered dose are in the United States of America accepted as almost diagnostic of an overactive gland yet such figures are found in a high proportion of normal individuals in Great Britain In both countries values below 20 per cent indicate hypothyroidism

Measurements of gland uptake during the uptake phase of iodine concentration are of more value and correlate well with the ultimate peak level attained This has influenced the development of several tests Miller *et al* (1951) showed that uptake measurements at any time between 3 and 7 hours were useful in differentiating normal from hyperthyroid gland function Ansell *et al* (1953) found that 4-hour uptakes below 40 per cent were seldom encountered in hyperthyroidism although higher uptakes occasionally occurred in euthyroid individuals while Beck *et al* (1956) concluded that 4 hours was the most useful time period McConahey *et al* (1956) favoured 6 hours and felt that uptakes at that time represented the most simple rapidly performed test which was most suitable for routine testing In their experience uptakes over 30 per cent at 6 hours indicated hyperthyroidism though some patients with toxic nodular goitre had lower values In general measurements of uptake made 4 or 6 hours after the test dose will be most convenient both for patients and observers

The value of ^{131}I tests of thyroid function has been enhanced by the observation made by several groups that the administration of l triiodothyronine in a dose of 100 micrograms daily for a week inhibits gland uptake in euthyroid individuals but not in those with exophthalmic goitre Two tracer tests are performed one before and one after the period of administration of l triiodothyronine By this means it is possible to obtain more precise diagnostic assistance

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time This technique involves one or two intravenous injections absolute standardiza tion of the dose in terms of its counting rate in air at a known distance from the counter (for calibration of the thyroid counts) and separately for calibration of the plasma concentration Normally the thyroid clearance rate is of the order of 0 5–3 5 litres per hour and over 5 litres per hour in hyperthyroidism

The thyroid clearance after one hour has been measured using an intravenous technique by Berson *et al* (1952) Coenegracht and Fraser (1955) found a good correlation between the results of this test and the T index derived from divided urine collections It can replace the latter and give a valid answer in a short space of time

The inconveniences of direct measurement of thyroid clearance are avoided by the use of the neck thigh ratio (Pochin 1950) This ratio essentially a clearance measurement depends upon the level of uptake after two hours related to the counting rate over the thigh the latter being a measure of the concentration of radio iodine in the body which has not been collected by the thyroid The ratio does not require knowledge of the exact dose given and no blood or urine samples need be taken The values obtained are dependent upon the exact counter arrange ments geometry and shielding used in any particular unit in Pochin s experience and under his conditions the ratio only occasionally exceeded 7 in patients without thyroid disease values may be over 40 in hyperthyroidism

This index is of value as a rapid screening test There are considerable advantages however in expressing results in terms of percentage of dose administered when this is done parallel measurements can be performed on other parameters such as uptake excretion or protein bound plasma radioactivity

Measurements of radioactive thyroid hormone

In a thyrotoxic individual the discharge of radioactive iodine from the gland starts within a few hours but it is longer delayed and of lesser degree in a euthyroid person Determination of the protein bound plasma radioactivity ($PB^{131}I$) has been shown to be of great value as a diagnostic index either when expressed as a percentage of the total plasma radioactivity (the conversion ratio) or as an absolute amount in terms of percentage of the administered dose per litre of plasma In thyrotoxic persons the conversion ratio is usually over 35 per cent and always over 50 per cent when measured at 24 hours whereas in normal individuals it seldom exceeds 35 per cent and is usually under 15 per cent (Clark 1955) In the assess ment of hypothyroidism the measurement is of no value

Determination of the 48 hour $PB^{131}I$ has been shown by Goodwin *et al* (1951) to be one of the best single tests in the diagnosis of hyperthyroidism and Wayne (1954) showed that of 343 cases of hyperthyroidism only 4 per cent showed levels below 0 4 per cent of the dose per litre of plasma Hughes and Miller (1956) found that total plasma activity levels were a useful and reliable test of thyroid function and stated that a value of 0 4 per cent or less was an almost certain indication of normal thyroid function Paley *et al* (1955) considered that the 48 hour $PB^{131}I$ was of value in thyrotoxicosis in a previously untreated patient but that if the patient had been treated clearance tests were less likely to be mis leading

Urine excretion tests

An indirect measurement of gland uptake is provided by the renal excretion of radioactive iodine The renal clearance rate for iodide is similar both for normal and for thyrotoxic individuals and the proportion of a given dose excreted is inversely related to the amount taken up by the gland Normal thyroid function is usually associated with an excretion of over 35 per cent of the dose after 24 hours but the test is insufficiently discriminating to be of much value

Keating *et al* (1947) originally showed that urinary assay over selected time intervals was a great deal more valuable

This principle has been developed and is now widely employed following the technique of Fraser *et al* (1953) Urine collection is subdivided into the periods 0–8 hours 8–24 hours and 24–48 hours Such a subdivision permits of the calculation of the index T from the formula —

$$\frac{(\text{0–8 hour excretion per cent of dose} \times 100)}{(\text{8–24 hour excretion per cent of dose})} \text{ (0–48 hour excretion per cent of dose)}$$

This index and two others also described can be used to cross check on the results for accuracy of urine collection and for the presence of renal disease The normal range of 0–48 hour urine excretion is from 35 to 70 per cent with the T index ranging from 3 to 13 In hyperthyroidism the 8–24 hour fraction is usually under 6 per cent the total excretion under 30 per cent and the T index over 17 The total excretion in hypothyroidism may be from 55–95 per cent and overlap with the normal range but the T index is always helpful and falls below the normal range

In many centres it is felt that the potential inaccuracy of urine collection and the occasional difficulty of interpretation of results because of concomitant renal or cardiac disease renders urinary tests of thyroid function unreliable However there is no doubt that in the hands of intelligent patients and co operative nursing staff useful information can be obtained with minimal inconvenience The intrinsic accuracy of measurements of the urinary concentration of radio iodine as contrasted with the potential inaccuracy of any estimations of the radio iodine content of the gland is such as to render urine excretion tests of value in the majority of cases Like estimations of blood or plasma radioactivity they make it possible for a patient s thyroid activity to be tested without any contact with counting apparatus and technical personnel an advantage of some importance when large numbers of patients have to be handled

Thyroid iodide clearance rates

Myant and Pochin (1949) and Keating *et al* (1949) at the Mayo Clinic first proposed that a direct measure of thyroid activity could be obtained by testing the thyroid clearance rate for iodide It is certainly an index of thyroid iodide utilization but as Riggs (1952) pointed out it is even less specific than uptake measurement as an index of hormone secretion This is because thyroid iodide clearance is determined in part by renal clearance of iodide as well as by the factors which influence uptake For many purposes however it is of value

Serial measurements of gland uptake are made about one hour after an oral dose of radio-iodine possibly earlier after an intravenous dose and the rate of thyroid uptake estimated and related to the observed concentration of plasma radio iodine at that

Concurrent or past administration of drugs

The most serious errors are due to the use of drugs or previous operative or isotope treatment Iodide in the form of cough mixture or iodine as Lugol s solution may depress thyroid uptake for some weeks in euthyroid persons and for a much shorter period in thyrotoxic individuals A qualitative test for urinary iodide such as that described by Fraser *et al* (1953) should be performed if abnormally low uptakes are noted The radiological contrast media used for cholecystography pyelography bronchography angiography and so on contain iodine and can depress thyroid uptake for varying periods up to months or even years (Foote *et al* 1952) Inquiry should always be made regarding the possibility of such investigations having been performed

Thyroid extract and thyroxine inhibit endogenous thyrotrophic hormone production and so diminish thyroid gland activity However it is possible to tell whether potentially active thyroid tissue is present or not by making uptake measurements before and after the injection of thyrotrophic hormone (T S H) Details are given later Antithyroid drugs prevent iodide binding by the thyroid gland the radio iodine is not retained in the gland and therefore tests during drug administration may show a falsely depressed uptake On withdrawal of the drug and particularly if it has been administered for a long time an induced deficiency has been caused and the gland may show a high uptake which is a reflexion of iodine deficiency This is similar to that in iodine deficient goitre (Stanbury *et al* 1954)

Iodine deficiency

Iodine deficiency accounts for the great variation in accepted normal ranges for thyroid gland uptake in different regions of the world Differences are due to variations in local dietary intake even when the latter may not be regarded as being pathologically low It has been shown that the abnormally high uptake of drug induced iodine deficiency can be restored to normal by pretreating the patient with iodide before testing the high uptake of hyperthyroidism is not affected by this procedure (Fraser 1956) Alternatively thyroid extract or thyroxine may be used in full doses for this purpose High uptakes due to iodine deficiency will be suppressed and will fall within the normal range but if they are the result of the thyrotoxic state they will remain unchanged (Werner *et al* 1952 Greer and Smith 1954) The simultaneous performance of a test measuring $PB^{131}I$ may avoid a false assumption of hyperthyroidism as the rate of appearance of radio active hormone in the plasma is often unaltered and the $PB^{131}I$ or the conversion rate remain normal This type of falsely high uptake may result from the use of drugs not usually recognized as having an antithyroid effect para aminosalicylic acid given to tuberculous patients (Macgregor and Somner 1954) or resorcinol applied to the skin (Bull and Fraser 1951) are examples

After previous thyroidectomy after radioactive iodine therapy and in some patients with lymphadenoid goitre a normal or subnormal thyroid gland uptake of radioactive iodine may be associated with a high level of $PB^{131}I$ This reflects a rapid discharge of the isotope from the gland because of a high turnover rate and occurs when relatively small numbers of residual follicles are functioning

Multiple tests of thyroid function

The best single tests for the detection of hyperthyroidism are the 48 hour protein bound plasma radioactivity the neck–thigh ratio the 4 hour gland uptake and the urine excretion in the 8–24 hour period On the other hand hypo thyroidism is best detected by the 48 hour gland uptake or full 48 hour excretion test with the calculation of the T index and K_t

Although a single test may be sufficient for diagnosis increased accuracy and reliability can be obtained if two tests such as the measures of gland uptake of radio iodine and of protein bound plasma radioactive iodine are used Ansell *et al* (1953) demonstrated that the combination of 4 hour uptakes and 48 hour $PB^{131}I$ was superior to other indices In a series of subjects both normal and frankly thyrotoxic Wayne (1954) showed that when the tests were in agreement this combination had an error of about 1 per cent In a further series of cases all of which had presented some diagnostic difficulty Crooks *et al* (1957) placed this figure at 3 per cent In this study the upper limit of normal gland uptake at 4 hours was taken as 45 per cent and of $PB^{131}I$ at 48 hours as 0 4 per cent per litre Werner (1955) also preferred a combination of tests but used the 24 hour gland uptake readings and the $PB^{131}I$ 72 hours after the dose had been given A complete and divided urine collection may also be combined with a $PB^{131}I$ measurement a routine in which the patient does not need to be brought into contact with counting equipment

In in patient practice a comprehensive study can conveniently include urine collection 4 hour and 48 hour uptake readings and 48 hour $PB^{131}I$ Out patients if intelligent should have a urine collection and 48 hour $PB^{131}I$ or if convenient a 4 hour or 48 hour gland uptake measurement If urine collection is undesirable a quick and reliable diagnosis can be made either from a neck thigh ratio reading alone or from the combination of a 4 hour uptake and a 48 hour $PB^{131}I$

Fallacious results in radio-iodine tests

These may be due (1) to technical imperfections in the measurements (2) to disturbances of radio iodine metabolism arising from disease other than thyroid disease or (3) to the concurrent or past administration of drugs

Technical errors

Technical errors are chiefly encountered in connexion with measurements of gland iodine uptake The geometry of the recording system must exclude all possibility of abnormally high counts from secondary backscatter radiation Body background must be excluded by suitable shielding procedures or preferably and more easily allowed for by making a separate count over the thyroid region with a lead shield occluding the thyroid area itself It is essential that care be taken to avoid any contamination of equipment or glassware Complete urine collections must be ensured and checked by the divided collection technique

Associated disease

Associated renal disease or heart failure with renal impairment may result in an abnormally high gland uptake of radio iodine because of the low renal clearance rate

Concurrent or past administration of drugs

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USE OF THYROTROPHIC HORMONE IN ASSOCIATION WITH RADIOACTIVE IODINE TESTS

A physician has often to decide whether the administration of thyroxine to a patient is really necessary and if the patient is already receiving thyroxine or thyroid extract conventional radioactive iodine tests are of little value For a decision the drug must be withheld for at least a month and sometimes even longer By the use of thyrotrophic hormone (T S H) however the presence of responsive thyroid tissue can be determined even while thyroxine or thyroid extract is being administered An untreated patient or one to whom thyroid has recently been given reacts to T S H by an increase in the neck uptake of radioactive iodine when compared with a similar and previous test carried out without T S H

Dosage

A single dose of five international units of T S H may be sufficient but it is better to give 10 units This should be administered either in one injection 24 hours before the second radioactive iodine test or alternatively given in three 8 hourly divided doses preceding the radio iodine test The latter procedure is probably preferable since the more prolonged and continuous gland stimulation is more likely to provoke a response than is the single injection

The thyroid response can be assessed either by a 4 hour or 24 hour uptake measurement or by the 48 hour urinary excretion or by the T index derived from a divided collection A significant response is indicated by an increase in the 24 hour gland uptake of more than 15 per cent of the total uptake or a similar reduction in the 48 hour urine excretion

✓The same test can be used to differentiate between primary and secondary hypothyroidism In primary myxoedema there is no response but in secondary hypothyroidism from hypopituitarism except in a few cases of very long standing where the thyroid gland has become atrophic preliminary administration of T S H can elicit an increased thyroid uptake of radioactive iodine The test is critically *discussed with full references by Bishopric et al (1955) It has also been shown* that the response to T S H is markedly impaired in struma lymphomatosa (Hashimoto s disease)

SERUM CHOLESTEROL IN THYROID DISEASE

Thyroid hormone increases the rate of hepatic biosynthesis degradation and intestinal excretion of cholesterol The net result in thyrotoxicosis is a fall in the serum cholesterol while in myxoedema there is a rise Unfortunately the normal range of the serum cholesterol is wide and different laboratories quote figures which vary with their technique and the number of normal estimations carried out Wooton and King (1953) stated that 90 per cent of normal individuals had serum cholesterol figures lying between 153 and 260 milligrams per cent and 99 per cent had figures between 123 and 324 milligrams per cent In a series of 150 thyrotoxic patients we found only 30 per cent with serum cholesterol levels below 150 milligrams per cent However all patients with values below 100 milligrams per cent were thyrotoxic The level almost always increased with treatment

In myxoedema the serum cholesterol is usually raised It is always above 200 milligrams per cent and may rise to 800 milligrams per cent In hypothyroidism secondary to pituitary hypofunction on the other hand relatively low levels are common and 50 per cent of one series of such patients had serum cholesterol figures below 200 milligrams per cent (van Arsdel and Williams 1956) It must be remembered that high levels of serum cholesterol occur in numerous other conditions for example diabetes mellitus the nephrotic syndrome obstructive jaundice xanthomatosis and pregnancy In these conditions however there is usually a disturbance of the ratios both of esterified to free cholesterol and of total cholesterol to phospholipids In hypothyroidism the normal ratios of these fractions are maintained (Peters and Man 1950)

In the past too little attention has been paid to lipid fractions other than the total serum cholesterol A high total serum cholesterol level is only specifically indicative of hypothyroidism if the cholesterol phospholipid ratio remains normal

A fall in serum cholesterol occurs with treatment in myxoedema and this can be used as a diagnostic therapeutic test

SERUM CREATINE AND URINARY CREATININE

In tests of disturbances of thyroid function there is undoubtedly a place for a simple objective measurement which correlates well with clinical diagnosis Griffiths (1951) suggested that this may be found in the fasting serum creatine level which he regarded as of diagnostic value in thyrotoxicosis especially when correlated with the basal metabolic rate He takes 0 6 milligram of creatine per cent as the upper limit of normal It should be remembered however that the serum creatine may be raised in disorders of muscle metabolism and in rheumatoid arthritis (Freeman and Mattingly 1956)

Urinary creatinine estimations are technically very simple and Friedman *et al* (1954) have shown a close correlation between the pigment-creatinine ratio (Ostow and Philo 1944) and the basal metabolic rate especially in thyrotoxicosis The increased creatininuria is not specific but it does diminish with antithyroid therapy unlike that from other causes such as carcinomatosis

THE USE OF ^{13} I

This short lived isotope with a half life of 2 26 hours has recently become available Its rapid decay enables studies to be carried out at short intervals and its radiation effects are of such short duration that it can be used in children and during pregnancy Either the uptake at 1 hour or the clearance at 1 hour by Berson s (1952) technique may be studied Its chief drawback in our experience is the difficulty of obtaining consistent readings Other observers have reported similar findings (Hanbury *et al* 1954)

THE RELATIVE VALUE OF TESTS OF THYROID FUNCTION

The tests reviewed measure different aspects of thyroid function radio iodine tests and the plasma protein bound iodine indicating the state of activity of the gland the basal metabolic rate and serum cholesterol measuring the hormonal effect on

tissue cells It is not surprising that they may not be in agreement In thyrotoxicosis radio iodine measurements of the 4 hour gland uptake and 48 hour plasma activity are most valuable although in certain circumstances other methods may have advantages Protein bound plasma activity for instance is technically speaking relatively difficult to measure and time consuming In myxoedema the 48 hour gland uptake and the T index can be relied upon Basal metabolic rate investigations even when carefully carried out do not possess the diagnostic accuracy of radio iodine criteria though they help to grade the severity of the disease Estimations of the serum cholesterol are of little diagnostic value in thyrotoxicosis but may be helpful in myxoedema In conclusion it can be stated that the use of the more simple and generally available tests with the essential clinical data will almost always lead to correct diagnosis and give a reliable guide to long term treatment

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CHAPTER 5

TREATMENT OF TOXIC AND MALIGNANT THYROID DISORDERS WITH RADIOACTIVE IODINE*

E E POCHIN

INTRODUCTION

THE APPLICATION of the radioactive forms of iodine to the problems of clinical medicine has progressed rapidly and continuously since these isotopes became available

The first form of radio iodine prepared by Fermi (1934) was of too short a half life for clinical use and the longer lived isotopes described in 1938 by Livingood and Seeborg were initially cyclotron produced in too small quantities to have therapeutic value although test doses revealed the increased uptake of the overactive gland (Hamil ton and Soley 1940) and a selective radio iodine concentration in thyroid carcinoma metastases (Keston *et al* 1942) The first substantial therapeutic dose of radio-iodine was given to a patient with thyroid carcinoma in 1943 (Seidlin Marinelli and Oshry 1946) although a 10 millicurie dose had been used in the attempted treatment of such a case in 1942 (Keston *et al* 1942) Patients with hyperthyroidism were being treated with cyclotron produced mixtures of ^{130}I and ^{131}I from 1941 (Hertz and Roberts 1942 Hamilton and Laurence 1942) and by ^{131}I from nuclear reactors from 1946 In the last ten years many hundreds of carcinoma patients and many thousands of hyper thyroid subjects have been treated by the use of radioactive iodine

In numerous respects however both of these forms of treatment are still developing particularly in their adaptation to varied clinical circumstances and in the selection of appropriate patients This chapter therefore must be concerned as much with the present problems and uncertainties as with their normal practice which has been fully described in a number of publications (Hyperthyroidism has been discussed by Fraser Abbatt and Stewart 1954 Horst and Kuhlencordt 1954 Blomfield *et al* 1955 and Chapman and Maloof 1955 Thyroid cancer has been considered by Coliez *et al* 1953 Rawson Rall and Robbins 1953 Pochin Cunningham and Hilton 1954 Hahn 1956 Hilton 1956 and Maloof Vickery and Rapp 1956)

RADIO IODINE THERAPY IN HYPERTHYROIDISM

Advantages

The aim is to give a dose of radio iodine which will irradiate the gland sufficiently to reduce its activity to normal without causing undue irradiation of any other part of the body Radioactive iodine is not distinguished from other iodine in its metabolism and is rapidly and highly concentrated in the gland between 70 and 90 per cent of the dose is usually taken up by the hyperthyroid gland within 12 hours of its administration and most of the remainder is excreted in the urine

Based on work undertaken on behalf of the Medical Research Council

It is therefore possible to deliver 8 000-9 000 rads to the gland without exceeding 10 or 20 rads of irradiation to most other body areas In this respect intense irradiation is more fully confined to the gland itself than during treatment with external radiotherapy a dose sufficient to cause a remission can more easily be given since relatively little irradiation extends beyond one millimetre from the gland tissue and the necessary dosage is not limited by risks of skin damage as with external radiation For the patient the treatment consists in a drink apparently of cold water with a further such drink a few months later if his thyroid remains over active It combines the merits of thyroidectomy in avoiding the need for continuing treatment and those of the antithyroid drugs in avoiding the necessity of operation

Disadvantages and contra indications

Radio-iodine does not however at the present moment appear to be the treatment of choice in all cases of hyperthyroidism for several reasons although different clinics vary in the weight that is attached to these reasons

Pregnancy

In pregnancy radio iodine administered to the mother enters the foetal circulation Pregnancy would therefore be accepted as a contra indication to radio-iodine therapy in all centres although the foetal thyroid itself collects iodine only during the last two trimesters

Childhood

In childhood at least to the age of 6 years it appears likely that the developing thyroid is unusually subject to malignant change following radiation (Duffy and Fitzgerald 1950 Simpson Hempelmann and Fuller 1955 Clark 1955) Radio iodine therefore should certainly not be used therapeutically in hyperthyroidism in children

Degree of radiosensitivity

Overactive glands differ considerably in radiosensitivity One patient may remain thyrotoxic after an amount of thyroid irradiation which in another patient is excessive and causes myxoedema This difficulty is not of great importance since the frequency of permanent myxoedema is a few per cent only in most series and does not differ greatly from that following subtotal thyroidectomy The number of doses needed is rarely more than 3 and averages of about 1 3 are most commonly reported

Large glands and tracheal narrowing

Effective treatment of thyroid overactivity is usually associated with a substantial reduction in the size of the gland most commonly to about half its initial size For very large glands, therefore or when the persistence of any goitre is undesirable surgery would be preferable Surgery would certainly be wiser in the presence of severe stridor or tracheal narrowing although in a few such cases treated with radio iodine we have not observed increase in respiratory difficulty even in the week after the dose when maximal radiation effects would be occurring in the gland

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* Based on work undertaken on behalf of the Medical Research Council

percentage of cases this sequel occurs If it proves to be carcinogenic in 5 per cent of cases after 20 years it will rarely be used except perhaps in elderly subjects or as a last resort If it is carcinogenic in 0 1 per cent of cases it is likely to be safer than either surgery or the prolonged use of antithyroid drugs and may become the usual treatment of choice There is not yet however and for about 10 years cannot be any basis whatever for answering quantitatively the question of how frequently radio-iodine therapy is carcinogenic in man and our use of it until then must remain as uncertain as many other clinical judgments At present it seems unjustifiable to use radio iodine as the treatment of choice in young patients and where other treatments are known to be safe but it would be equally unjustifiable to withhold it where the alternatives are hazardous and particularly in patients with post operative recurrent thyrotoxicosis or in those over 45 years of age with severe thyroid disease for whom the prognosis after 20 years is in any case doubtful It will be appreciated that even this opinion implies that the late risk of radio iodine treatment can be approximately estimated which is certainly not true At present some clinics use radio iodine as the treatment of choice in all adults except during pregnancy and perhaps with large toxic nodular goitres many would use it in patients over 45 years of age some would only give it to patients for whom alternative treatment was contra indicated and rarely to patients under 45 years of age

Technique of radio-iodine therapy in hyperthyroidism

In a hyperthyroid patient selected as suitable for radio iodine therapy the following steps are normally involved (1) Preliminary drug treatment if any is judged to be necessary (2) A decision as to the thyroid irradiation required and usually a test dose of radio iodine to determine the dose needed to produce this (3) A therapeutic dose and control of any symptoms which may be provoked by it (4) Any necessary control of persisting hyperthyroid symptoms in the month or two following the therapeutic dose and before its full effect is obtained (5) Later assessment of thyroid state to exclude continued overactivity or the development of myxoedema

Certain problems may arise at each stage and the methods adopted in different clinics vary

Preliminary treatment

If a patient is receiving antithyroid drugs they should be discontinued for a short period before giving radio iodine An interval of a few days to a week is normally used and enables the gland s capacity for concentrating and retaining iodine to be restored The test dose will then estimate adequately the uptake of the subsequent therapeutic dose

If the patient has not been receiving antithyroid drugs it is usually unnecessary to give them (*but see* Fraser Abbatt and Stewart 1954) Nevertheless with severe hyperthyroidism or in advanced thyrocardiac disease it is wise to control the gland s overactivity with preliminary antithyroid drugs before radio iodine and so avoid the risk of exacerbation of symptoms during the week or so after the therapeutic dose Antithyroid drugs can be discontinued when adequate control

Problems in treating toxic nodular goitres

Toxic nodular goitres are often stated to be more difficult to treat by radio iodine or to require larger doses than glands which are uniformly hyperplastic This difficulty however does not prevent the effective control by radio iodine of most of these cases (Cook Jones and McCullagh 1955)

Dosage

The estimation of the correct dose of radio iodine (in millicuries) for the required irradiation of the gland (in rads) presents certain problems apart from the need for specialized equipment for the necessary measurements The irradiation produced will depend on the proportion of the dose concentrated in the gland the volume of gland through which it is distributed the uniformity of its distribution and the length of time during which it remains in the gland Several of these factors may be estimated by a preliminary test dose of radio iodine (*see below*) The estimate so obtained is unlikely to be of great accuracy but the large variations in thyroid radiosensitivity make it unnecessary to seek a high precision in individual cases These measurements necessitate hospital attendances for about a week prior to treatment their omission must involve some increased uncertainty as to the size of dose required

Side effects

Therapeutic radio iodine administration has been shown to cause a rise in the serum protein bound iodine concentration during the week following the dose presumably due to thyroxine and other metabolites liberated from irradiated thyroid cells Increased thyrotoxicity during this period is not usually observed but may constitute a slight risk in severe thyrocardiac disease Local tenderness of the gland is common but not severe Acute radiation sickness used to occur during treatment by mixtures of ^{130}I and ^{131}I but is normally not detectable after ^{130}I

Possibility of malignancy

The only major objection to the wide use of radio iodine as the treatment of choice in most cases of hyperthyroidism is that it is not yet certain that malignancies may not be induced by it As in the radiotherapy of any benign condition the possibility of late carcinogenesis perhaps following irradiation by 20 years indicates the need for caution in the use of this treatment initially

At present some hundreds of patients are surviving 12 years and some thousands 8 years after treatment No subsequent malignancies of the thyroid or of closely adjacent structures have been recorded the two cases of leukaemia reported as following radio iodine therapy of hyperthyroidism (Pochin Myant and Corbett 1956 Abbatt Farran and Greene 1956) barely exceed the number to be expected by chance The risks however cannot be known for some 10 years or more since an appreciable malignancy incidence may develop after a long latency

Experience in other fields has shown that malignant changes may be expected after radiative destruction of various tissues and animal experiment has shown that radio iodine treatment can in fact be followed by thyroid neoplasms in the rat thiouracil treatment being similarly carcinogenic in this species The problem however is not whether radio iodine treatment is carcinogenic in man but in what

The therapeutic dose

For the therapeutic dose admission to hospital appears unnecessary in most cases since there is usually no subsequent exacerbation of symptoms and certainly no likely hazard from the uncontrolled rejection of the small proportion of the dose excreted in the urine In cases however with severe hyperthyroidism observation in hospital during the week following the therapeutic dose is a wise precaution Some nausea perhaps with diarrhoea may occur a few days after the dose and the gland may become tender to palpation but general symptoms of radiation sickness or acute hyperthyroidism are most unlikely

Course subsequent to the therapeutic dose

The therapeutic dose appears to cause a progressive reduction in thyroid activity reaching a maximal effect after about 2 months this depression decreasing some what in the following month (Larsson 1955) If this behaviour is usual it follows that (1) patients with initially severe hyperthyroidism may require additional therapy during the weeks after the dose (2) transient hypothyroidism may develop at 6 weeks after the dose and (3) final assessment of the effect of the first dose can be made after 3 or 4 months although if the patient is still hyperthyroid after 1½–2 months further treatment is likely to be needed

Most patients need no additional therapy following the dose which may improve symptoms within 2–3 weeks For those previously receiving antithyroid drugs and in whom a return of symptoms might be hazardous iodides or anti thyroid drugs can be given from a few days after the dose Lugol s iodine or iodides have been advocated on the grounds that their action is sufficiently prolonged to cover the necessary interval and that involution of the gland following radiation damage should theoretically involve less risk of carcinogenesis than the pituitary stimulation of the gland which may occur from excessive blockage of thyroxine formation by antithyroid drugs Experimental evidence in animals suggests that if radio iodine is followed by antithyroid drugs it is more frequently carcinogenic than if not so followed

No satisfactory test has been found which will determine soon after a therapeutic dose whether this dose will restore normal thyroid activity and it is usually necessary to rely on clinical and radio iodine assessment made at 12 or more weeks If additional treatment is necessary the dose is usually determined on an arbitrary basis Most clinics in fact give a further dose of between one third and two thirds of the original dose varying the proportion according to the degree of remaining hyperthyroidism

The possibility of post irradiation myxoedema should be remembered and that it may be only transient Thyroid preparations should be given initially only for a few months and then discarded unless hypothyroid symptoms return with their withdrawal or reduction

Conclusion

Radio iodine treatment of hyperthyroidism is simple and efficient although it still requires greater accuracy and earlier assessment of the need for furtherdosage If by 1965 it has proved to be safer than expert thyroid surgery it may become the treatment of choice for most patients

has been obtained or a euthyroid condition established The test dose is then given about five days later

Determination of dose size

Hyperthyroid glands differ widely in their radiosensitivity An average radiation intensity throughout the gland of 8 000–9 000 rads is usually sufficient to reduce thyroid activity to normal In any particular patient higher or lower intensities may be required or thought preferable In an elderly patient with thyrocardiac disease the need to control thyroid activity with a single dose may justify an increased risk of inducing myxoedema and so call for 10 000–12 000 rads In a younger patient with moderate post operative recurrent thyrotoxicity and to whom several doses could be given without inconvenience the risk of myxoedema might be reduced by aiming at 6 000 rads from a first dose Toxic nodular goitres appear to require higher intensities for full control than diffusely enlarged glands and higher initial doses are often used when such cases are treated (Cook Jones and McCullagh 1955)

The size of the therapeutic dose required to produce a given intensity of irradiation may be estimated from the behaviour of the preceding test dose A carrier free test dose of 30–50 microcuries is given and measurements made of the percentage of this present in the thyroid at say 1 3 5 and 7 days after its administration so that the maximal uptake and iodine retention in the gland can be estimated A clinical estimate of thyroid size may be supplemented by a map of the radio iodine distribution in the neck (*or see* Franco and Quina 1956) From the uptake the gland size, the discharge rate and the physical properties of ^{131}I the necessary dose size can be calculated small corrections being possible for the gamma radiation contribution in large glands and for the greater efficacy of radiation delivered during a short period than during a long one The therapeutic dose is given as soon as the measurements following the test dose are completed

In some clinics no test dose is given and the size of the therapeutic dose is determined solely on the clinical estimate of gland size and on whether heavy or moderate radiation is required When this is done the published results do not appear to be worse than when detailed measurements on a test dose are made the frequency of myxoedema and of the therapeutic doses required is not increased This may be because individual differences in radiosensitivity overshadow the importance of varying uptake or discharge rates it seems analogous to the usual penicillin or salicylate dosage on a body weight basis blood levels only being required as a refinement or in certain cases At present it would appear preferable to base the therapeutic dose on a preceding test dose whenever the latter can be given without difficulty though justifiable to omit it under special circumstances

Since variations in iodine discharge rate are much larger than those in uptake a test dose followed only for 24 hours for determination of uptake is of little value Errors in the determination of gland size are very large whether by mapping or by palpation and a radiological assessment following intracervical injection of air has been proposed (Franco and Quina 1956) Most clinics would hesitate to use single doses of more than about 20 millicuries even if such were required to produce the necessary radiation intensity

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(5) Re-examination 6–8 weeks later by which time myxoedema will normally have developed and administration of a therapeutic dose if adequate uptake is demonstrable in tumour tissue

(6) Repetition of therapeutic doses at intervals if the above is established until no iodine-concentrating tissue can be detected or until an adequate response has been obtained

(7) Thyroxine or thyroid preparations are given once uptake has developed but with interruption of such treatment during the 4 weeks preceding any radio-iodine dose since tumour uptake is suppressed during and for 2–3 weeks after thyroid replacement therapy

(8) Ultimate follow up examinations for example with annual radio iodine test doses to exclude the recurrence of iodine-concentrating tissue

In a typical favourable case the abolition of all detectable functioning carcinoma tissue may be achieved in 2–3 years from thyroid ablation and after a total dosage of 1 500–1 800 millicaries of ^{131}I There are however wide differences of response from patient to patient and of procedure in different clinics

Selection of patients

Most clinics would regard tumours as suitable for attempted radio iodine treatment if consisting largely of colloid filled follicles and unsuitable if their cells were without either follicular or papillary arrangement or of the Hurthle cell type Good responses may also be obtained if follicles are scattered throughout a mainly papillary pattern or are small and without colloid in their lumens When the tumour structure is wholly papillary as judged from a biopsy sample radio iodine is often said to be without value and such tumours may sometimes be well treated by successive removal of local recurrences Our experience however indicates that radio iodine treatment following thyroid ablation may be valuable in some of these cases and that it should certainly be attempted if surgery fails even though follicles are absent and the tumour is of a papillary type

On the other hand it is rarely of value to use radio iodine in undifferentiated carcinomas without cell arrangement These tumours often spread so rapidly that they cause death within the 3 months that would be necessary for the induction of myxoedema and the development of iodine uptake following thyroid ablation If a wholly or partly anaplastic tumour is judged to be unsuitable for surgery or radiotherapy and is progressing only slowly in a young and otherwise well patient it may sometimes be justifiable to proceed with thyroid ablation and tests of uptake and response to radio iodine The results are however usually unfavourable and it is certainly unjustifiable to subject the majority of patients with anaplastic tumours to a terminal myxoedema even if external radiotherapy has been unsuccessful or inapplicable It is of course important to remember that in some follicular carcinomas the follicles are so small and their lumina so slight or absent that the resulting closely packed rosettes of cells may be mistaken for solid masses of cells of undifferentiated type

Method of ablation

In any patient selected for radio iodine treatment abolition of normal thyroid function is a necessary first step The choice between total thyroidectomy and radio iodine as methods of achieving thyroid ablation will be determined by the

RADIO IODINE THERAPY OF THYROID CARCINOMA

When radio iodine is used in the treatment of thyroid carcinoma the problems and limitations are entirely different from those involved in the therapy of hyper thyroidism The isotope is used not as an alternative but as the only treatment of clinical value risks of inducing further malignancy are secondary Moreover the doses required for full control of the tumour may be very large so that radiation effects on other body tissues are likely to limit the amounts that can be given in any one dose

The main problems again however are the selection of suitable patients the preparation necessary before radio iodine can be started the conduct of treatment and assessment of its completion Although the technique of this form of therapy has progressed considerably since it was first used in 1942 there is still much uncertainty as to the best procedures to adopt at the several stages In some clinics the isotope is used according to a much less well defined programme than would appear desirable

The possibility of treating a thyroid carcinoma with radio iodine depends upon the isotope being sufficiently well concentrated and retained in the tumour tissues to cause destructive local irradiation without causing undue radiation of other essential body structures The concentration of radio iodine in turn depends upon the tumour cells resembling normal thyroid tissue in their metabolism sufficiently to be capable of synthesizing thyroid hormone and of their being under suitable stimulus to do so

It is now clear that anaplastic thyroid tumours rarely concentrate radio iodine to a therapeutically useful extent though histologically differentiated ones do so quite commonly as might be expected if histological and biochemical differentia tion ran parallel It is also found that relatively few thyroid tumours concentrate iodine strongly in any case unless stimulated to do so by the cessation of normal thyroid function and the development of myxoedema

It is usually impossible to select patients by an initial test for tumour uptake of radio iodine and the decision is commonly made on histological grounds direct evidence of iodine concentration in tumour tissue only being sought after the initial abolition of thyroid function Even then the efficiency of uptake does not in itself indicate whether the tumour will be sufficiently radiosensitive to respond to the radio iodine which it concentrates and the response to a therapeutic dose may be the soundest guide to the value of this treatment

Technique of radio-iodine therapy in carcinoma

For the above reasons the following steps are commonly adopted

(1) Examination of excised tissue to establish the diagnosis and the histological nature of the tumour

(2) Attempted radical resection of the tumour in all instances where this appears at all likely to be practicable

(3) For inoperable but differentiated thyroid tumours ablation of the thyroid either by a total thyroidectomy or by an initial therapeutic dose of radio-iodine

(4) Examination 6–8 weeks later for evidence either of radio iodine uptake in tumour tissue or of incomplete ablation of the thyroid a therapeutic dose being given if either is found

a normal level of thyroxine metabolism If myxoedema is present and tumour uptake of radio iodine clearly demonstrable therapy should be started with a radio iodine dose of about 150 millicuries Many clinics however would require a high uptake certainly exceeding 20 per cent of the dose concentrated in tumour tissue and would allow myxoedema to continue until a sufficiently high uptake was demonstrable before giving the first radio iodine dose they often give drugs of the thiouracil group in high dosage or thyrotropic hormone by injection to ensure maximal pituitary or other stimulation of the tumour to concentrate iodine before using radio iodine therapeutically

This difference in procedure—either in starting treatment when any uptake is demonstrable or in requiring high uptake before treatment—arises from several circumstances on which information is still required

(1) If the radiation of a tumour (by radio iodine or otherwise) depresses its powers of concentrating further radio-iodine for many months then the greatest possible uptake must clearly be ensured before any therapeutic dose is given If uptake is only depressed for a few weeks then early treatment and repeated doses can be given

The question is difficult to answer unequivocally since a radio-iodine dose which destroys half the tumour cells will necessarily halve the uptake of an ensuing dose even after full recovery of concentrating power in the surviving cells It seems likely that at least in some tumours the full power is recovered in a few weeks

(2) It is clear that many tumours will only concentrate iodine strongly under the stimulus of myxoedema and presumably as a result of the increased thyrotropic hormone released from the pituitary in this condition It is not clear however whether when a patient is already myxoedematous drugs of the thiouracil group will achieve any further stimulation of tumour uptake If so and the evidence on this point is slight such drugs should be given from the time of ablation and stopped a few days before each test If not the development of full myxoedema would imply that the tumour uptake was already fully stimulated and further delay might allow only increased tumour growth The same doubt attaches to the value of thyrotropic hormone to stimulate tumour uptake during established myxoedema

(3) It is also uncertain whether prolonged thyrotropic hormone stimulation of a thyroid carcinoma in man may not favour its spread and enlargement as well as its iodine uptake If this is so as it is for certain thyroid tumours in animals prolonged continuation of myxoedema may be hazardous to the patient as well as distressing

(4) No measurement can estimate the total mass of iodine concentrating tumour tissue particularly where palpable masses may be partly necrotic or fibrotic or after treatment It is thus impossible to know whether a low uptake is due to a small mass of actively functioning tumour which would favour immediate treatment or to a large mass with inefficient uptake which might favour delay if this efficiency could be improved Multiple drill biopsies with autoradiography might answer this question in an individual patient but these are rarely justified or practicable

(5) Even if the radio iodine uptake per gramme of tumour tissue and so the irradiation to be expected from a given dose could be estimated we have at present inadequate information about the radiosensitivity of different tumours A well differentiated tumour of good uptake may respond less well than a poorly differentiated one of weaker uptake For this reason the response to radio iodine of a tumour which has developed its maximal uptake affords the best criterion for starting radio iodine therapy

At present therefore some clinics would allow myxoedema perhaps combined with antithyroid drug treatment to continue unless the tumour was concentrating a considerable percentage of a test dose others would only do so if little or no

circumstances of the case Surgical removal of the gland will normally be preferable in the following circumstances

(1) If there appears to be any likelihood that the tumour may prove to be wholly removable

(2) If a large mass of tumour tissue can readily and safely be removed from the neck during the thyroidectomy

(3) If the trachea is severely compressed but can be freed at the time of operation The risk of tracheal obstruction due to oedema following a large dose of radio iodine appears to be small but may occur in cases with considerable initial stridor For such patients operation is best and the trachea should be freed even if total thyroidectomy proves impossible When stridor is severe and no adequate resection can be made a temporary tracheotomy may be desirable before radio iodine ablation

(4) If the trachea is moderately compressed by a rapidly enlarging tumour since radiotherapy may not become effective in reducing tumour mass before the development of myxoedema 3 months later

On the other hand radio iodine ablation will usually be preferable

(1) If neck structures are likely to have been disturbed by previous operation and *radio-iodine therapy as well as by the presence of tumour tissue so that a total thyroi*dectomy is unlikely to be completed without substantial risk to parathyroids or recurrent laryngeal nerves

(2) If one vocal cord is already paralysed

(3) If the thyroid is unduly involved with vascular tumour tissue or is densely adherent to the trachea so that total thyroidectomy will be difficult to achieve safely

If radio iodine is used an ablation dose of 80 millicuries will usually destroy almost all normal thyroid tissue without causing an unduly severe local reaction although tenderness of the gland and local oedema commonly occur during the ensuing week Some nausea and symptoms attributable to moderate radiation sickness may occur during the 24 hours following the dose and general symptoms may sometimes recur a few days after the dose at a time when it can be shown that radiative destruction of thyroid tissue is liberating iodine metabolites into the circulation For these reasons and to ensure proper control of the highly radioactive urine during this period it is desirable that the patient should be in hospital during the week following an ablation or other therapeutic dose No major problems are likely to arise except very occasionally cervical oedema threatening the airway of a patient who had severe stridor before the dose

Requirements for starting radio-iodine therapy

Myxoedema is unlikely to develop until 10–12 weeks after thyroid ablation It is however useful to re examine the patient at 6–8 weeks after radio iodine ablation or at 2 weeks after total thyroidectomy partly to determine whether iodine uptake has already developed in the tumour but chiefly to see whether all thyroid tissue has in fact been ablated and to use radio iodine to complete the ablation if it has not It is common to find a trace of such tissue still functioning and concentrating 0 5–1 per cent of a test dose and a therapeutic dose almost always *needs to be given at this stage*

On re examination 6–8 weeks later myxoedema has usually developed except when a large mass of tumour tissue is already functional and capable of maintaining

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selective uptake was demonstrable at tumour sites once myxoedema had developed Most would start treatment for a tumour estimated as concentrating 0 1 per cent of a test dose per gramme of tumour few would start if the estimate was 0 001 per cent per gramme

We have no information as to the lag which may occur between the onset of myxoedema and the development of iodine uptake in tumour tissue Tests should probably be made for at least 3 months before the possibility of abandoning radio iodine treatment Even if little uptake is then demonstrable one therapeutic dose should perhaps be given since the risks of giving it are negligible, and it may be followed by a response in a radiosensitive tumour concentrating only small amounts of radio iodine

Conduct of treatment

Most tumours responding to radio iodine require repeated large doses and decisions in the conduct of treatment are concerned with the size of individual doses the necessary interval between doses and the criteria for discontinuing treatment

Dosage

Individual doses of between 100 and 200 millicuries are usually given if repeated dosage is intended After such doses radiation sickness is usually slight and blood changes transient a depression of the lymphocyte count commonly lasts for about 3 weeks after the dose The intervals between doses vary widely in different clinics but there is some evidence that tumour cells surviving one dose recover their power of iodine concentration within 4 weeks although the power appears to be lost for the week or two following the therapeutic dose It would seem desirable therefore to continue doses of say 150 millicuries at 6–8 week intervals until no remaining tumour tissue is demonstrable In practice this would involve prolonged hypothyroidism since thyroid extract itself depresses iodine uptake in the tumour for several weeks after its administration and so needs to be discontinued for 3 or 4 weeks before each dose A suitable procedure is to give doses 6 or 8 weekly until a steady diminution of tumour mass is demonstrable and then to widen the interval between doses progressively provided that the diminution continues

Estimation of radio iodine uptake in tumour mass

It is not possible to estimate directly the total tumour mass and to adjust treat ment so as to ensure its progressive diminution However it is possible to estimate in most cases the total radio iodine uptake in the tumour mass and in many cases this value appears to run parallel with the tumour mass itself The correlation between total tumour uptake and total tumour mass in any one patient is not invariable but it appears to give a useful guide to progress in many patients and is usually more helpful than clinical or radiological estimates of the size of metas tases alone The tumour uptake can be estimated directly by suitably calibrated gamma radiation counting over body positions at which tumour tissue is present A more indirect but simpler estimate may be derived from the proportion of the dose which is found to be protein bound per litre of plasma at a given interval after the dose or from the proportion which is excreted in the urine only after a delay indicating its retention in iodine concentrating tissue

All these methods and that correlating uptake with tumour mass require further investigation It is most important that the destruction of iodine-concentrating tumour tissue should be estimated reliably so that the optimal size and interval of doses can be determined in each patient It is also valuable to know accurately the interval needed for recovery of uptake both after radiation and after thyroxine and the interval necessary for bone marrow recovery between doses

Complications during treatment

In a patient whose carcinoma has developed good iodine uptake several events may interrupt the plan of repeated therapeutic dosage until no further iodine concentrating tumour tissue can be detected

(1) Metastases with good uptake may nevertheless fail to respond or cease to respond even to frequent and large radio iodine doses This appears more likely to occur if the total tumour mass is already large and inoperable at the start of treatment

(2) New functioning metastases will occasionally develop and progress despite continued treatment

(3) Any metasta es that do not concentrate iodine may extend or new deposits of undifferentiated type may develop This complication seems to be infrequent

(4) Death may be caused by remaining tumour tissue We lost one case owing to retroperitoneal haemorrhage from tumour tissue in which the total uptake on repeated doses had been decreasing

(5) Treatment may have to be discontinued owing to bone marrow depression This is often regarded as a likely complication of radio iodine treatment of any thyroid carcinoma In our experience a substantial fall in blood count occurred only in patients with bone metastases In such ca es it usually developed about a year after treatment and affected erythrocytes and platelets as well as leucocytes In patients without bone metastases the blood count has rarely been much affected except during the week or two immediately following each dose This has only once caused delay or interruption of treatment It is our experience that for a limited period radio iodine is clinically valuable in many patients with bone metastases In those without radio iodine treatment can usually be pressed without fear of marrow aplasia and it should be so pressed if possible until total tumour destruction has been achieved

Re-examination after treatment

Even if this state has apparently been reached it is still desirable that the patient should be re examined at intervals both clinically for recurrence and for any re appearance of abnormal radio iodine uptake At present we give an annual test dose large enough to allow detection of a tumour recurrence concentrating 0 01 per cent of the dose so using 10–25 millicurie doses 4 weeks after discontinuing thyroxine administration It is too early to judge for how long such re examinations should be continued and it is much too early to know whether patients in whom no remaining traces of iodine concentrating tumour tissue can be detected will remain free from recurrence without further treatment

Summary

Radio iodine therapy has an obvious clinical value in a substantial proportion of patients with inoperable and well differentiated thyroid cancers and should be attempted as the treatment of choice in most of these cases

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CHAPTER 6

ENDOCRINE FACTORS IN THE SYNDROME OF DIABETES MELLITUS

P J RANDLE

INTRODUCTION

EXPERIMENTAL and clinical observations during the past 30 or 40 years have implicated a number of hormones in the syndrome of diabetes mellitus These include the pancreatic hormones insulin and glucagon the pituitary hormones growth hormone corticotropin and prolactin certain adrenal steroids and the thyroid hormone The present account of the subject is not so much concerned with establishing that they have a role in the diabetic syndrome but rather in assessing their relative importance This account is far from complete and no attempt has been made to discuss for example the importance of the thyroid hormone obesity or heredity in the diabetic syndrome

Clinical forms of diabetes mellitus in man

Essential diabetes presents in a severe form and in a mild form of an obese or non obese nature

When found to be of secondary nature the disease can be seen in the following conditions pancreatic disease (for example after pancreatectomy carcinoma of pancreas) acromegaly hypercorticoadrenalism hyperthyroidism phaeochromocytoma lipoatrophy

Although different clinical forms of diabetes are recognized there are no generally accepted terms to describe them A simple classification of the different forms is given above In this classification the term essential diabetes is used to separate the vast majority of cases in which diabetes occurs as a disorder in its own right from those in which it occurs as a feature of some other well recognized disease The severe cases comprise those patients in which insulin treatment is essential if normal weight is to be maintained and ketosis avoided and the mild cases are those in which insulin treatment is not necessary The severe cases correspond to those classified by Himsworth (1949) as insulin sensitive and by Lawrence (1951) as insulin deficient The mild obese cases correspond to those described by Himsworth as insulin insensitive and by Lawrence as lipoplethoric The present classification is preferred since it avoids references to insulin sensitivity and to aetiology which are in many cases unknown

The pancreas and diabetes

Two hormones of significance in diabetes have so far been produced from the pancreas These hormones insulin and glucagon have been isolated in pure form

and their chemical structure is now known (insulin—Ryle *et al* 1955 glucagon—Bromer *et al* 1956) Insulin has been isolated only from the pancreas (Best Jephcott and Scott 1932) but glucagon may occur in certain parts of the alimentary tract as well as in the pancreas (Sutherland *et al* 1949)

INSULIN

The significance of insulin in diabetes needs no substantiation and all forms of the disorder can be attributed to deficiency of insulin action It may arise in four ways from defective secretion of insulin by the pancreatic islets from excessive inactivation of insulin from humoral antagonism to insulin action and from some inherent abnormality of cellular metabolism so that the cell is no longer capable of responding to insulin The last possibility is of purely theoretical interest at the present time but the other mechanisms have all been implicated in the diabetic syndrome

An analysis must therefore include estimates of the rates of secretion and inactivation of insulin The evidence on these points has been derived from studies of the histology and insulin content of the pancreas the blood level of insulin activity, the rate of degradation of labelled insulin and from observations on the clinical features and insulin sensitivity of different forms of diabetes

Histology and insulin content of the pancreas

A number of histological changes have been noted in the pancreatic islets in human and experimental forms of diabetes These include atrophy hypertrophy degranulation and hydropic degeneration of the β cells and hyalinization fibrosis and thickening of the basement membrane in the pancreatic islets Hydropic degeneration would appear to be due to infiltration of the pancreatic islets with glycogen (Toreson 1950) The recent studies of Hartroft and Wrenshall (1955) suggest that the β cell granules may represent or contain stored insulin They have compared the index of β cell granulation and insulin extractable from the pancreas in 80 diabetic and 86 non diabetic patients Statistical analysis of their results appears to show conclusively that there is a direct correlation between the extract able insulin of the pancreas and the index of β cell granulation Hartroft and Wrenshall consider also that some of the insulin may be present in the form of submicroscopic granules and that the proportion of this may be increased in the diabetic pancreas

The only histological change which has been shown to be of functional signifi cance in diabetes is β cell degranulation Richardson and Young (1948) sectioned the whole pancreas from a cat which had recovered spontaneously from meta hypophyseal diabetes Histological examination failed to reveal a single normal β cell yet despite the gross histological changes in the islets the animal was not diabetic Hartroft (1956) emphasized that the pancreatic islets may be normal in cases of human diabetes and that degeneration accompanied by degranulation of the β cells was only found in 5–15 per cent of adult diabetics and hyalinization with or without fibrosis and basement membrane thickening in 25–40 per cent The vast majority of juvenile diabetics (in which the onset of diabetes occurs before 17 years of age) showed extensive degranulation of the β cells

Islet tissue in diabetic subjects

Maclean and Ogilvie (1955) estimated the quantity of islet tissue in diabetic subjects in which the pancreatic islets did not exhibit hyalinization or significant fibrosis The proportion of β cells in the islets was reduced in diabetics of all ages but most severely when diabetes appeared during the growing period In the latter group the weight of the pancreas the number of islets per pancreas and the pro portion of islet tissue were also reduced

Lower extractable insulin in diabetics

Wrenshall Bogoch and Ritchie (1952) assessed the extractable insulin content of the pancreas in 213 human subjects of which 64 were diabetic control experi ments suggested that the insulin content did not alter in the period between death and removal of the pancreas The extractable insulin in the diabetics as a group was much lower than in the non diabetics dying from the same cause and it was also very low in growth onset diabetics (onset before 17 years of age) but rose in those of maturity onset to a maximum level of one half that of the non diabetic controls Extractable insulin was extremely low after death in diabetic coma The cases classified by these authors as growth onset or as maturity onset would presumably correspond to those cases classified here as severe or as mild essential diabetes

The relationship between the insulin content the histology of the pancreas and its capacity to secrete insulin has yet to be established The insulin content of the pancreas will be determined by the rates of formation and secretion of insulin

Insulin content of blood plasma

The information about diabetes so far obtained from estimations of insulin activity* in blood plasma is limited and inconclusive The methods available for its assay are neither sufficiently accurate nor specific to provide an acceptable estimate of the insulin content of the plasma Moreover even if it could be measured the level in the plasma would not provide an estimate of the rate of secretion for the level of insulin in the blood is regulated both by rate of secretion and the rate of removal by the tissues

The activity of insulin which may involve binding by tissues on which it acts (Stadie 1954) would appear to depend upon its concentration in blood or extra cellular fluid Thus diabetes associated with a fall in plasma insulin would indicate insulin deficiency whilst diabetes with normal or increased levels would indicate inhibition of insulin action

A number of methods are now available for the detection and assay of minute amounts of insulin Some of these methods have been shown by statistically controlled investigation to merit the dignity of the term assay but only with pure insulin Not one of these methods has been shown to be capable of estimating accurately the insulin content of crude preparations of the hormone or of blood plasma

The requirements of a bio assay for the estimation of a hormone in biological fluids have been discussed by Segaloff (1953) He suggested that such an assay

* The term insulin activity refers to the amount of insulin producing an effect equivalent to that of a given volume of plasma in the bio assay without prejudice as to whether the effect of the plasma is due solely to insulin

procedure must be specific reproducible from laboratory to laboratory sensitive statistically sound simple rapid and inexpensive In Table I these criteria are applied to existing methods for the assay of insulin activity in blood plasma

TABLE I

EVALUATION OF METHODS FOR THE ASSAY OF INSULIN ACTIVITY IN BLOOD PLASMA[1]

Method based on	*Specific*	*Reproducible*[2]	*Sensitivity milli units (1 milliunit = 10^{-3} unit*	*Statistically sound*	*Simple and rapid*	*Inexpensive*	*Author*
(*a*) Blood sugar response of							
Adrenodemedullated hypophysectomized rats	Not known	Not known	1	Not known	No	No	Gellhorn *et al* (1941)
Adrenodemedullated alloxan diabetic hypophysectomized rats	Not known	Not known	0 125	Not known	No	No	Anderson *et al* (1947)
Alloxan diabetic hypophysectomized adrenalectomized rats	No	No	0 05	Not known	No	No	Bornstein (1950)
Alloxan diabetic hypophysectomized mice	Not known	Not known	0 01	Not known	No	No	Yenerman *et al* (1953)
Alloxan diabetic hypophysectomized rats	Not known	Yes	2–3	Not known	No	No	Beigelman *et al* (1956) Randle (unpublished data)
(*b*) Uptake of glucose *in vitro* by isolated rat diaphragm	No	No	0 005	Yes	Yes	Yes	Groen *et al* (1952)
			0 01				Vallance Owen and Hurlock (1954)
			0 10	λ=0 20–0 40[3]			Randle (1954b 1955 1956)

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Reprod c bl f o n labo tory t l bo tory
λ = i d of p ec n = st d d d t of po ts b t eg e n l / l p of g n l

Only two of these have so far been applied to the insulin activity of blood plasma in diabetes (1) the blood sugar response of alloxan diabetic hypophysectomized adrenalectomized rats (ADHA rats) and (2) the uptake of glucose *in vitro* by the isolated rat diaphragm Discussion will be restricted to these two methods

ADHA rats—blood sugar response

The assay of insulin by ADHA rats was based upon the fall of blood sugar level over a period of one hour following subcutaneous injection of insulin or plasma

(Bornstein 1950). A full statistical appraisal of the method has not been published though the blood sugar response was shown to bear a linear relation to log. dose of insulin. It was said to detect as little as 0·05 mU* of insulin.

The method was applied to the insulin activity of plasma from normal or diabetic subjects by Bornstein (1950), Bornstein and Trewhella (1950, 1951) and Bornstein

TABLE II

INSULIN ACTIVITY OF PLASMA FROM NORMAL AND DIABETIC PATIENTS

Type of patient	*Plasma insulin activity milliunits insulin/ml. of plasma (1 milliunit = 10^{-3} unit)*	*Assay*	*Author*
Normal	0·10 (fasting) 0·34 (after glucose 2–2½ hours)	ADHA rat	Bornstein (1950)
	0·10–3	Rat diaphragm	Willebrands and Groen (1956)
	0·01–0·10 (fasting) 0·10–0·80 (after glucose 1 hour)	Rat diaphragm	Vallance-Owen and Hurlock (1954)
	1–20 (after glucose 2–2½ hours)	Rat diaphragm	Randle (1954b)
	0·01–10	Rat diaphragm	Gray, C. H. (personal communication)
Essential diabetes			
Severe			
untreated treated	None detected 0·22	ADHA rat	Bornstein and Trewhella (1950) Bornstein and Lawrence (1951a and b)
untreated or treated	Normal level	Rat diaphragm	Groen *et al.* (1952) Willebrands and Groen (1956) Randle (unpublished observations)
poorly controlled with insulin	None detected	Rat diaphragm	Vallance-Owen *et al.* (1955)
well controlled	0·10		
Mild obese	0·22	ADHA rat	Bornstein and Lawrence (1951a and b)
	Normal level	Rat diaphragm	Willebrands and Groen (1956) Randle (unpublished observations)
	0·03–0·50 (fasting) 0·20–0·60 (after glucose 1 hour)	Rat diaphragm	Vallance-Owen *et al.* (1955)
Acromegaly			
Diabetic treated with insulin or non-diabetic	None detected — plasma contained hyperglycaemic substance	ADHA rat	Bornstein and Lawrence (1951b)
	Markedly increased	Rat diaphragm	Randle (1954b) Willebrands and Groen (1956) Candela (1956)

* 1 mU = 1 milliunit = 10^{-3} unit

and Lawrence (1951a and b) The insulin activity of plasma from normal people was 0 1 mU/ml (fasting) to 0 34 mU/ml (2–2½ hours after oral glucose) Plasma from untreated cases of severe essential diabetes 2–2½ hours after oral glucose lacked insulin activity whereas plasma from cases treated with insulin showed approximately two thirds normal insulin activity Plasma from untreated cases of mild obese diabetes possessed approximately two thirds normal insulin activity while plasma from patients with acromegaly and untreated diabetes was found to raise the blood sugar level of ADHA rats Bornstein and Lawrence (1951b) concluded that cases of essential diabetes could be subdivided into two groups with and without available insulin in the plasma and that these groups were identical with the two broad groups of diabetics separable on a clinical basis

Criticism of results—The results obtained with this procedure can be criticized on several grounds The assay procedure can only be regarded as qualitative since standard doses of insulin were not included in the assays of plasma insulin activity The specificity of the blood sugar response of ADHA rats when used to assay insulin in plasma was not established either by multi dose assays or by a full study of possible interfering factors Plasma from acromegalic patients with diabetes had a hyperglycaemic effect in ADHA rats whereas plasma from severe cases of essential diabetes in addition to being devoid of insulin activity was capable of rendering them resistant to the action of insulin in a subsequent test It is to be inferred from these observations that the plasma of some of these diabetic patients contained factors capable of antagonizing the action of insulin in the ADHA rat and that therefore the ADHA rat assay as used by Bornstein and his colleagues does not necessarily measure the insulin content of plasma This latter point was emphasized by Bornstein and Lawrence who were careful to draw conclusions only about the available insulin content of plasma The principal results obtained by Bornstein and his colleagues are summarized in Table II

These interesting results obtained with the ADHA rat have yet to be confirmed Several investigators have attempted to develop the ADHA rat assay but without success finding the animals too delicate for assay purposes (Peden 1955 Salter and Best cited by Peden 1955 Gray personal communication and Randle unpublished observations)

Uptake of glucose by isolated rat diaphragm

The second method of assay which has been applied to the insulin activity of diabetic plasma is based on the glucose uptake of isolated rat diaphragm (Groen Kamminga Willebrands and Blickman 1951 Vallance Owen and Hurlock 1954 Randle 1954b 1955) A statistical appraisal of this method recently published shows that a straight line assay for insulin can be based upon the uptake of glucose *in vitro* by the isolated rat diaphragm The precision of the assay was not very great (Randle 1956b)

The results of assays of insulin activity in plasma from normal people and from diabetics are summarized in Table II and show that those from normal human plasma vary considerably Values have been obtained ranging from 0 1 to 0 8 mU/ml (Vallance Owen and Hurlock 1954) 0 1 to 3 mU/ml (Willebrands and Groen 1956) 1 to 20 mU/ml (Randle 1954 and unpublished observations) and

0 01 to 10 mU/ml (Gray—personal communication) the reason for these discrepancies is not apparent

As regards the results in diabetics Groen *et al* (1952) found that the plasma from patients with severe diabetic acidosis was devoid of insulin activity whereas the plasma from other types of diabetic whether severe or mild treated or untreated possessed essentially normal insulin activity Similar results have been obtained by Randle (unpublished observations) Vallance Owen Hurlock and Please (1955) claimed that plasma from severe essential diabetics treated with insulin was devoid of insulin activity if the patients were poorly controlled at the time when the blood was collected but that it possessed essentially normal activity if the patients were well controlled In mild obese diabetics in the fasting state plasma insulin activity appeared to be greater than normal but failed to rise after oral glucose This was in contrast to the normal subject in whom plasma insulin activity showed a marked increase after glucose (Vallance Owen and Hurlock 1954 Vallance Owen 1956)

Plasma insulin activity in acromegaly

Randle (1954a 1955) observed a marked increase of plasma insulin activity in acromegaly even when it was associated with diabetes which had been treated with insulin and this observation has been confirmed by Willebrands and Groen (1956) and by Candela (1956) In keeping with this Randle and Young (1956) found in the intact cat that treatment with growth hormone for several days led to marked increase in the insulin activity of the plasma In the depancreatized cat receiving insulin treatment with growth hormone only led to an increase in plasma insulin activity when the daily dose of insulin was increased during the period of treatment Randle and Young concluded that treatment with growth hormone in the intact cat leads to an increase in the plasma level of insulin and to an increased rate of insulin secretion and suggested that the enhanced plasma insulin activity in acromegaly might also reflect an increased rate of insulin secretion brought about by growth hormone They emphasized that these conclusions were drawn from a comparison of the effects of growth hormone treatment on plasma insulin activity in normal and depancreatized cats and that they were independent of conclusions concerning the specificity of the assay procedure

Insulin plasma effect on glucose uptake

The specificity of the rat diaphragm assay for insulin in plasma leaves much to be desired The belief that the stimulating effect of plasma from normal animals upon glucose uptake is due solely to the action of insulin in the plasma rests upon the observation that plasma from depancreatized animals deprived of insulin is devoid of stimulating activity (Groen *et al* 1952 Randle 1956a Vallance Owen 1956) However this evidence is suspect since substances with inhibitory activity towards the uptake of glucose or to the response to insulin of the isolated rat diaphragm have been identified in the plasma of severely diabetic animals including alloxan diabetic rats (Bornstein and Park 1953 Randle 1955) meta hypophyseal diabetic cats (Randle and Young 1956) severe cases of essential diabetes in man (Vallance Owen Hurlock and Please 1955) and depancreatized cast (Vallance Owen 1956) Four point assays with insulin and with plasma from normal people normal rats and acromegalic patients have revealed significant

differences between the slopes of regression lines for insulin and for plasma (Randle unpublished observations) The widely differing values which have been reported for insulin activity of normal human plasma (*see* Table II) also cast doubt upon the specificity of the method The conclusion must be drawn that the rat dia phragm assay does not provide a specific estimate of the insulin content of plasma Whether the insulin like action of plasma upon the uptake of glucose by the isolated rat diaphragm is due principally to an action of insulin in the plasma or to a non specific effect of plasma protein as Bornstein and Park (1953) have suggested remains to be established In the author s view an approach most likely to provide an accurate estimate of the content of the plasma insulin has recently been provided by Weitze and Hagedorn (1954) and Beigelman *et al* (1956) These authors attempted partial purification of insulin in plasma prior to assay by acid alcohol extraction (Weitze and Hagedorn 1954) or by plasma protein fractionation (Beigelman *et al* 1956)

Classification of diabetes on insulin sensitivity

Himsworth (1949) suggested on the basis of a study of clinical features and sensitivity to insulin that the form of diabetes classified here as severe essential diabetes results from deficiency of insulin whereas the form classified as mild obese essential diabetes results from factors other than deficiency of insulin Lawrence (1951) emphasized this view of the aetiology of human diabetes and suggested that severe cases of essential diabetes may eventually exhibit absolute deficiency of insulin the insulin content of the pancreas in these two types of diabetic would lend support to this view Within the limits of the experimental technique dis cussed above estimations of plasma insulin activity by means of the ADHA rat assay are also in keeping with this view On the other hand studies with the rat diaphragm technique have failed to provide clear evidence either for or against for insulin activity has been detected in the plasma from both types of case Although Vallance Owen *et al* (1955) failed to detect insulin activity in the plasma of poorly controlled essential diabetics treated with insulin their results do not indicate that the plasma lacked insulin for they demonstrated the presence of a factor which prevented the action of insulin

Inactivation of insulin

The components of a system which regulates the inactivation of insulin have been described by Mirsky *et al* (1956) in a long series of papers They comprise an enzyme or enzyme system which inactivates insulin (insulinase) and a factor which inhibits insulin inactivation (insulinase inhibitor) The activity of insulinase has been demonstrated in tissue extracts or slices by its ability to render insulin biologically inert (Mirsky and Broh Kahn 1949 Tomizawa *et al* 1954) and to release ^{131}I from the protein bound form with insulin labelled with ^{131}I (Mirsky Perisutti and Dixon 1954 1955 Tomizawa *et al* 1954) Insulinase has the properties of an enzyme or enzyme system (Mirsky *et al* 1954) and appears to be proteolytic (Vaughan 1954 Tomizawa *et al* 1955) while its partial purification has also been achieved (Vaughan 1954) Mirsky *et al* (1955) believe that insulinase is specific for the breakdown of insulin whereas Tomizawa and Williams (1955) claim that α corticotrophin casein glucagon and growth hormone

are also attacked The activity of insulinase has been demonstrated in extracts of many tissues In the rat liver and kidney showed the greatest insulinase activity while none could be detected in the brain some activity was demonstrable in skeletal muscle (Mirsky and Broh Kahn 1949)

^{131}I *labelled insulin*

The degradation and distribution of insulin *in vivo* has been studied by many workers using insulin labelled radioactively with ^{131}I The proportions of ^{131}I in the supernatant (non protein bound) and precipitate (protein bound) after the treatment of blood or tissues with trichloracetic acid have been used as an index of insulin degradation The objection to the use of ^{131}I labelled insulin on the grounds that it may not be fully active physiologically and that it may be biologically distinguishable from unlabelled insulin has to some extent been overcome by the observation that the administration of unlabelled insulin will reduce the rate of breakdown of labelled insulin In the rat and dog labelled insulin after administration is especially found in the liver and kidney little or none is found in the brain whilst muscle exhibits intermediate activity (Haugaard Vaughan Haugaard and Stadie 1954 Elgee Williams and Lee 1954) The latter authors found a somewhat similar distribution of ^{131}I labelled insulin in man The distribution of radioactivity following the injection of labelled insulin corresponds closely to that of insulinase activity In rats the high content of radioactivity in the kidney is associated with the appearance of labelled degradation products of insulin in the urine (Haugaard *et al* 1954) while nephrectomy or hepatectomy leads to a reduced rate of degradation of labelled insulin (Elgee and Williams 1954)

An insulinase inhibitor has been identified in liver and other tissues of the rat by Mirsky Simkin and Broh Kahn (1950) The activity of this inhibitor which appears to be competitive in nature has been demonstrated *in vivo* and *in vitro* (Mirsky Simkin and Broh Kahn 1950 Mirsky and Perisutti 1955)

In a preliminary account of the activity of insulinase in liver obtained at biopsy from diabetic patients Mirsky (1956) stated that it was significant that greater insulinase activity was detected in liver samples from diabetic patients than in samples from non diabetic controls

Welsh *et al* (1955 1956) studied the degradation of ^{131}I labelled insulin in diabetic and non diabetic patients They found that following the administration *in vivo* of ^{131}I labelled insulin the ^{131}I persists in protein bound form in the plasma from diabetic patients for a longer period than in the plasma from non diabetic controls Similar observations were made when ^{131}I labelled insulin was added to normal and diabetic plasma *in vitro* When liver slices or diaphragms from rats were incubated with ^{131}I labelled insulin *in vitro* in the presence of diabetic serum less labelled insulin was bound by the tissue than with normal serum They concluded that the disappearance of insulin from the plasma and its degradation by tissues may be delayed in diabetes and that the plasma may contain some factor capable of binding insulin and preventing its uptake by the tissues It is to be inferred from this observation that the increased activity of insulinase in diabetic liver *in vitro* and as noted by Mirsky is not necessarily accompanied by an enhanced rate of destruction of insulin *in vivo*

Antidiabetic sulphonamide derivatives in relation to insulin

Janbon Lazerges and Metropolitanski (1942) reported that *p* amino sulphonamido isopropyl thiodiazole was hypoglycaemic in man and in experimental animals In recent years great interest in this and other sulphonamide derivatives has been aroused by reports of their effectiveness in controlling the symptoms of some cases of diabetes (Franke and Fuchs 1955 Bertram Bendfeldt and Otto 1955 and also Reports 1956)

Sulphonamide derivatives—which will be referred to collectively as such and not by individual names—would appear to be effective only in those diabetics in which insulin treatment is not essential to prevent ketosis

Three theories have been propounded to explain the hypoglycaemic and anti diabetic activity of sulphonamide derivatives Loubatieres (1946) believed that they act by stimulating the release of insulin from the pancreatic islets but Holt *et al* (1954) believed that they act by destroying the α cells of the pancreatic islets and so depressing glucagon production and secretion Mirsky *et al* (1956) reported that sulphonamide derivatives administered to rats depressed the activity of liver insulinase and they further observed inhibition of insulinase activity in rat liver extracts when sulphonamide derivatives were added *in vitro* The concentration of sulphonamide derivative needed to demonstrate inhibition of insulinase *in vitro* was however much greater than that found in the blood of animals exhibiting hypoglycaemia Furthermore the dose of sulphonamide derivative required to produce α cell necrosis (Holt *et al* 1954) was greater than that needed to provoke hypoglycaemia or to control diabetes The mechanism of the hypoglycaemic and antidiabetic action of sulphonamide derivatives is thus not yet established, but at the present time the most likely mechanisms would appear to be by stimulation of insulin secretion or inhibition of insulin destruction or both

Wrenshall and Best (1956) remarked upon the basic similarities in pattern between the presence or absence of appreciable quantities of extractable insulin in the pancreas at autopsy in their groups of cases of human diabetes and the effectiveness or ineffectiveness of antidiabetic sulphonamides in the cases of other workers Taken in conjunction these observations imply that antidiabetic sulphonamide derivatives are effective only in those cases in which appreciable quantities of endogenous insulin are *available that is in mild essential diabetes* In such circumstances antidiabetic sulphonamide derivatives might act by stimulating insulin secretion by inhibiting insulin destruction (sparing insulin) or by some other mechanism Thus no conclusions about diabetic aetiology can at present be drawn from studies with antidiabetic sulphonamide derivatives though they may prove a most useful tool when their mechanism of action is known

Summary

The evidence summarized suggests a number of steps in the metabolism of insulin and these are represented diagrammatically in Table III Theoretically interference with the metabolism of insulin at any of these stages could lead to diabetes mellitus So far only the insulin content of the pancreas the insulin activity of blood plasma the rate of degradation and binding of insulin have been studied at all in human diabetes

TABLE III
INSULIN METABOLISM

β cells of pancreatic islets

Synthesis of insulin

Insulin storage	(β cell granules)

INSULIN SECRETION

Blood plasma

Plasma insulin	⇌ Bound form of plasma insulin

INSULIN BINDING

Tissues

Insulin in tissues ⟶ Inactivation or degradation of insulin

Insulin action

The enclosed features of insulin metabolism represent those which have been studied in human diabetes

GLUCAGON

The idea that the pancreas might secrete a substance capable of intensifying the diabetic state originated in the observation that the insulin requirements of alloxan diabetic and metahypophyseal diabetic animals were reduced by total pancreatectomy (Thorogood and Zimmerman 1945 Marks and Young 1939) This substance may be identified with glucagon the hyperglycaemic glycogenolytic factor of the pancreas Mirsky (1952) however believes that in alloxan diabetes the reduced insulin requirements after pancreatectomy result from defective protein absorption In the rat and dog a diabetogenic effect of glucagon has recently been reported by Davidson Salter and Best (1956) though large doses were apparently necessary

In man insulin requirements after total pancreatectomy are substantially less than those of many cases of severe essential diabetes and from this it might be inferred that the pancreas secretes a substance intensifying human diabetes There is however no convincing demonstration that in human diabetics under controlled conditions of food intake pancreatectomy leads to a reduced insulin requirement

Kenny (1955) determined the glucagon content of the pancreas in diabetic and non diabetic patients at autopsy and found no abnormality in diabetes Bornstein and Trewhella (1951) and Bornstein and Lawrence (1951b) detected a hyperglycaemic factor in the blood of acromegalic patients while Bornstein Reid and Young (1951) identified hyperglycaemic activity in blood from the portal vein of cats made diabetic by treatment with growth hormone This hyperglycaemic

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for age at the time of the onset of their diabetes The possibility that hypersecretion of growth hormone might be responsible both for over rapid rate of growth and onset of diabetes is one for consideration This explanation however seems unlikely for Young (1944) found that crude preparations of growth hormone which were diabetogenic in the adult cat and dog did not induce diabetes in the kitten or puppy Furthermore pituitary gigantism unlike acromegaly is said not to be associated with an increased incidence of diabetes

(2) Miller Hurwitz and Kuder (1944) reported that mothers who give birth to abnormally large offspring show an increased incidence of diabetes in later life It has been suggested that the abnormally large offspring and subsequent onset of diabetes are due to hypersecretion of pituitary growth hormone during preg nancy More recently Abaza Varroud Vial and Rombauts (1953) described a condition which they have named Syndrome de Young in which maternal obesity and hyperlactation are associated with abnormally large offspring and the development of diabetes In 1949 Cotes *et al* discussed the increase of milk yield under certain conditions through the action of growth hormone and it has been suggested that this syndrome is a manifestation of hypersecretion of growth hormone during pregnancy and lactation Jackson (1954) on the other hand has observed that the offspring born to acromegalic mothers are not abnormally large Further more he believes that the fathers of abnormally large offspring are more prone to develop diabetes in later life He considers that hypersecretion of growth hormone is unlikely to account for the abnormally large offspring and the late onset of diabetes in the mother in such cases In the rat treatment with purified growth hormone has yet to be shown to increase litter size (Cotes 1954)

Procedures for the assay of growth hormone activity in blood have been described by Segaloff (1955) and Gemzell Heijkenskjold and Strom (1955) They have yet to be shown to be specific and have not so far been applied to a study of blood levels of growth hormone in human diabetics On the other hand increased blood levels of growth hormone activity have been noted in pregnant rats (Conto poulos and Simpson 1956)

Summary

Growth hormone has yet to be seriously implicated as a factor of prime import ance in the onset of essential diabetes Nevertheless although hypersecretion of growth hormone may not be responsible for the abnormally large offspring born to prediabetic mothers the possibility remains that it may occur during pregnancy and be a factor in the subsequent onset of diabetes

THE ADRENAL CORTEX AND DIABETES

A diabetogenic effect from the therapeutic use of cortisone and of corticotrophin has been demonstrated in man (Conn 1950 Bishop and Glynn 1952 Cohn and Kolinsky 1953 Bookman *et al* 1953) In the vast majority of cases the diabetes was only temporary though permanent diabetes did follow the administration of corticotrophin in the case described by Bishop and Glynn A significant loss of sensitivity to insulin has been noted by Zimmerman Parrish and Alpert (1953) in 4 of 17 patients treated with corticotrophin These observations are in keeping

activity may well be glucagon though Sirek and Best (1956) obtained evidence which suggested that the hyperglycaemic activity noted by Bornstein Reid and Young may have been due to an adrenergic substance and not glucagon

THE PITUITARY GLAND AND DIABETES

Three pituitary hormones—growth hormone prolactin and corticotrophin—have been shown to possess diabetogenic activity in experimental animals (Cotes Reid and Young 1949 Houssay *et al* 1955 Ingle Li and Evans 1946) This activity includes the ability to reduce sensitivity to insulin in normal and hypophysectomized animals (de Bodo and Altszuler 1955 Young 1936) Little is known of the importance of prolactin in human diabetes while corticotrophin will be considered in connexion with the adrenal cortex

Hypophysectomy and intensity of human diabetes

The investigations of Houssay and his colleagues between 1924 and 1932 showed conclusively that removal of the pituitary or its anterior lobe increases sensitivity to insulin in intact animals and ameliorates the diabetes of totally depancreatized animals The hypersensitivity to insulin and amelioration of diabetes in patients in whom panhypopituitarism develops and coexists with diabetes suggests a similar role for the pituitary gland in human beings Recent observations in diabetic patients following hypophysectomy have amply confirmed this Thus Luft *et al* (1955) and Kinsell Lawrence and Weyland (1956) noted a substantial reduction in insulin requirements in diabetics submitted to surgical hypophysectomy This effect of hypophysectomy would appear to be due to deprivation of corticotrophin thyrotrophin and growth hormone It is well established that hypocortico adrenalism and hypothyroidism in association with diabetes may lead to increased sensitivity to insulin and reduced insulin requirements However reduced activity of the thyroid and adrenal cortex after hypophysectomy can only account for a small part of the change in the diabetic state Thus both groups of authors have noted substantially reduced insulin requirements in hypophysectomized diabetics even after treatment with thyroid cortisone and testosterone or oestrogen It would seem reasonable to attribute this residual effect of hypophysec tomy to the absence of pituitary growth hormone

Growth hormone and essential diabetes

The increased incidence of diabetes in acromegaly associated in many instances only with the active phase of the disorder leaves little room for doubt that growth hormone is diabetogenic in man A convincing demonstration of the diabetogenic action of ox growth or pig growth hormone in man has not yet been reported but this is likely to be due to species differences in growth hormone (Kinsell 1955 Shorr *et al* 1955 Wilhelmi 1955 Knobil 1955 Knobil and Greep 1956)

The possibility that a pituitary factor now to be identified as growth hormone might be responsible for the onset of cases of essential diabetes has excited comment ever since Young (1937) demonstrated that permanent diabetes could be produced in dogs by a short period of treatment with anterior pituitary extract Two observations have suggested such a possibility

(1) White (1939) reported that many diabetic children were over height

COMPLICATIONS OF DIABETES MELLITUS

Retinal and renal lesions

The coexistence of retinopathy and intercapillary glomerulosclerosis is well established (Becker *et al* 1954) and they may well have a common aetiology The controlled investigations of Wilson Root and Marble (1951) and Dunlop (1954) appear to have established beyond possible doubt a relationship between poor control in diabetes and the incidence of complications It might be inferred from such an observation that diabetic complications arise from the operation of say hyperglycaemia but this is not necessarily so There is evidence for example that hypoglycaemia leads to reduced sensitivity to insulin (Somogyi 1949) and that this is due to the activity of a humoral factor of pituitary–adrenal origin (Bornstein 1953 Bornstein and Park 1953) In some cases of poorly controlled diabetes (brittle diabetics) alternate periods of hypoglycaemia and hyperglycaemia occur and the latter might be due to the activity of the humoral factors of pituitary adrenal origin whose release has been stimulated by hypoglycaemia or even by insulin in the absence of manifestations of hypoglycaemia

Abnormal hormonal factors

A number of authors have suggested that the complications of diabetes may result from abnormal secretion of hormonal factors by the pituitary and adrenal glands Thus diabetic retinopathy has been observed to appear during pregnancy in non hypertensive diabetics and to disappear following delivery (Lawrence 1948 Becker 1952) and retinal capillary aneurysms have been observed to appear in non diabetic patients during intravenous corticotrophin therapy and to disappear following cessation of treatment (Naquin cited by Becker *et al* 1954) Rich (cited by Becker *et al* 1954) observed intercapillary glomerulosclerosis in a non diabetic patient following prolonged corticotrophin therapy At autopsy increased incidence of abnormalities of the adrenal cortex have been noted in diabetics with intercapillary glomerulosclerosis It has also been reported that diabetics with retinopathy have excreted excessive amounts of free oxysteroids in the urine (Becker *et al* 1954) There are claims that lesions resembling those of retinal capillary aneurysms and intercapillary glomerulosclerosis may be produced in alloxan diabetic rabbits by cortisone administration (Becker *et al* 1954) Diabetic retinopathy has been noted in Cushing s syndrome and in acromegaly though as McCullagh and Alvisatos (1956) have emphasized diabetic retinopathy is not more frequent in these disorders than in ordinary diabetes

In a case reported by Poulsen (1953) diabetic retinopathy completely disappeared following the development of severe panhypopituitarism Luft *et al* (1955) and Kinsell *et al* (1956) reported on the results of hypophysectomy in diabetics with severe retinal and renal disease Their experience suggested that hypophysectomy may arrest the progress of diabetic retinopathy and nephropathy Wortham and Headstream (1954) carried out bilateral total adrenalectomy in diabetics with retinopathy and renal disease and noted some improvement following operation

Patterson (1956) believes that diabetic cataract may result from impaired utiliza tion of glucose by the lens in diabetes since high fat–casein–fructose diets possibly

with the incidence of diabetes in Cushing s disease and the observation that it may disappear following the removal of an adrenal cortical tumour

Effects of bilateral total adrenalectomy

Wortham and Headstream (1954) Martin and Wilson (1954) Hamwi (1954) and Conn cited by Kinsell (1955) studied the effects of bilateral total adrenalectomy in cases of essential diabetes Wortham and Headstream noted a reduction in insulin requirements following adrenalectomy but other authors have failed to detect any change in insulin requirements All patients of course received substitution treatment with cortisone or hydrocortisone but Martin and Wilson noted that their insulin requirements were governed by the dose of cortisone The different results can probably be explained on this basis

Addison s disease as a complication

Addison s disease complicating diabetes is known to be associated with increased sensitivity to insulin In terms of hyperglycaemia and glycosuria the diabetes may retain its former severity (McCullagh and Alvisatos 1956)

Output of corticosteroids

The urinary excretion of glycogenic cortico ids or corticosteroids in well controlled essential diabetes is normal (Venning and Browne 1947) or possibly reduced (Talbot *et al* 1951) Chow Becker and Silber (cited by Becker *et al* 1954) noted reduced urinary excretion of oxysteroids in diabetics without complications but increased urinary excretion of free oxysteroids in diabetics with retinopathy McArthur Sprague and Mason (1950) and Stowers (1951) noted increased output of cortico steroids in diabetic acidosis and this is rapidly reduced to normal levels with insulin treatment Kalant (1955) observed that the increased urinary output of corticosteroids in the alloxan diabetic rat was not associated with increased pro duction of corticoids by the adrenal glands *in vitro* and suggested that an increased urinary output of corticosteroids does not necessarily reflect increased production by the adrenal cortex It is to be inferred from this that the increased output of corticosteroids in diabetic acidosis in man may not reflect increased secretion of adrenal steroids

Glycogenic steroids, adrenal steroids and hydroxysteroids

Venning (1946) observed an increased urinary output of glycogenic steroids in months 1–3 and 4½–5½ of pregnancy while Gemzell (1953) and Bush (1953) obtained evidence which suggested that in normal pregnancy the levels of 17 hydroxysteroids are increased in peripheral blood Hoet (1954) reviewed the considerable evidence which implicates pregnancy as a factor in the onset of essential diabetes and considered that the hypersecretion of adrenal steroids during pregnancy might well play an important role The possibility of hypersecretion of growth hormone during pregnancy may also be of significance in this connexion

Summary

Apart from its possible role in the diabetogenic effect in pregnancy the adrenal cortex does not appear to be a prime factor in the onset of essential diabetes in man

GENERAL CONCLUSIONS

It is now generally conceded that the vast majority of cases of severe essential diabetes lack insulin and that this is primarily the result of defective production and secretion by the pancreatic islets None of the recent work presented here contradicts this view but it should nevertheless be emphasized that this applies only to established cases of diabetes We have no explanation as yet of the onset of diabetes itself or of its complications Evidence has been presented which suggests that abnormal activity of the pituitary and adrenal cortex may be operative in some of the complications but without producing the usual signs of hyperactivity as seen for example in acromegaly or Cushing s disease The possibility has been considered that a period of hyperactivity of the pituitary or adrenal cortex might be responsible for the onset of essential diabetes but in the author s opinion the evidence for this belief does not bear critical examination There is the possibility too that in established diabetes over activity of the pituitary and adrenal cortex may be an effect of insulin treatment and not an inherent abnormality of the disease

The principal hindrances to the advance in our knowledge of the hormonal factors in the syndrome of diabetes are largely technical Reliable and specific methods for the estimation of insulin and insulin antagonists in tissues and biological fluids would help to solve many of the problems In some instances up to the present the same procedure has been used to detect insulin activity and insulin antagonists in the same sample of serum It is clear that existing methods are not adequate The most fruitful approach would appear to lie in methods for the isolation of insulin and insulin antagonists from blood prior to assay

Of the mode of onset of diabetes and the factors responsible very little is known In the obese group of diabetics obesity itself is undoubtedly of importance Heredity has been implicated but an explanation of its mechanism in biochemical terms is lacking There have been suggestions that abnormal metabolites such as alloxan or abnormal products of purine metabolism having a toxic action upon the β cells may be produced in the body and be responsible for the onset of diabetes but the evidence is largely inferential It is clear that information about the onset of diabetes can only be obtained from a study of the onset of diabetes and at present we have no information for instance about the level of blood insulin or insulin antagonists in very early cases of diabetes

In the author s opinion three lines of approach are indicated in future research in diabetes First the development of satisfactory and specific methods for the estimation of insulin and other hormones in the blood to provide information about their chemical form as well as their concentration Secondly more emphasis on the study of the early and the prediabetic patient than on established cases of the disease Lastly renewed efforts should be made to detect substances in the blood or urine of early diabetics which are toxic to the β cells of the pancreatic islets or capable for example of enhancing the activity of insulinase

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providing metabolites which can be utilized in the absence of insulin prevented the appearance of cataracts in alloxan diabetic rats (Patterson 1955)

Summary

It would appear that the development of pituitary or adrenal insufficiency may arrest the progress and possibly even improve retinal and renal complications in diabetes Whether this results from better control of the diabetes effected by the operation or from removal of some pituitary or adrenal factor with a direct and harmful influence on the retina or kidney has yet to be established

Insulin resistance

The occasional occurrence of severe insulin resistance in diabetes necessitating the daily administration of several hundred or thousand units of insulin is well known and may be associated with cutaneous manifestations of allergy The antigenicity of insulin is well established such antibodies are capable of neutra lizing the action of insulin and of inducing diabetes (Maloney and Coral 1955) The serum of patients exhibiting severe insulin resistance has been shown to be capable of preventing the hypoglycaemic action of insulin (Lowell 1942 Lerman 1944 de Filippis and Ianaccone 1952 Schon *et al* 1955) and of preventing the *in vitro* action of insulin on the synthesis of glycogen by the isolated rat diaphragm (Marsh and Haugaard 1952) This serum anti insulin factor has been shown in some instances to have the properties of an antibody (Lerman 1944) and to be located in the γ globulin fraction of the serum (de Filippis and Ianaccone 1952 and Schon *et al* 1955) In keeping with this is the observation that the insulin resistance and serum anti insulin factor may disappear following treatment with corticotrophin (Kleeberg Diengott and Gottfried 1956 Marsh and Haugaard 1952) Lowell (1942) believes that the serum factor responsible for insulin resistance is distinct from that responsible for the cutaneous manifestations of allergy

Field and Stetten (1956) reported on an insulin antagonist in the serum of patients exhibiting insulin resistance which they believe is not an antibody The factor which was located in the α globulin fraction of the serum prevented an *in vitro* action of insulin upon the synthesis of glycogen by the isolated rat diaphragm but it disappeared rapidly from the serum of insulin resistant diabetics following vigorous treatment with insulin It was devoid of insulinase activity and as it was not present in the serum of patients treated with corticotrophin or in the serum of an insulin resistant acromegalic it was not likely to indicate activity of growth hormone or adrenal steroid

Bornstein and Park (1953) and Bornstein (1953) identified a factor in the serum of alloxan diabetic rats capable of antagonizing the *in vitro* action of insulin upon the uptake of glucose by the isolated rat diaphragm They presented evidence that the inhibitor was a lipoprotein and formed from or under the influence of growth hormone and cortisone This inhibitor has not been identified in the serum of diabetic patients though it has been demonstrated in that of patients receiving insulin shock treatment It would appear to be distinct from the insulin antago nists detected in the serum of insulin resistant diabetics

GENERAL CONCLUSIONS

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CHAPTER 7

ENDOCRINE ASPECTS OF OVERNUTRITION AND UNDERNUTRITION

G C KENNEDY AND R A McCANCE

INTRODUCTION

THE SUBJECTS of endocrinology and nutrition cannot be adequately studied in isolation The nutritional state of an animal affects both the synthesis of hormones and the response of the tissues to them Conversely a primary change in endocrine balance may profoundly affect the nutritional state both by its effect on appetite and upon the utilization of food This is obvious in the case of thyroid or pan creatic activity but perhaps less generally appreciated in the case of the pituitary gland (Samuels 1947 Ingle 1951 Meites 1953) Moreover there is increasing recognition that control of both the intake of food and of some aspects of expen diture of energy are closely integrated by the hypothalamus with the function of the anterior pituitary gland and thereby of other endocrine organs

FRÖHLICH S DISEASE AND ITS EXPERIMENTAL ANALOGUES

The experimental approach

The fact that destruction of the pituitary alone was not necessarily followed by obesity was first clearly demonstrated experimentally by Smith (1927 1930) He compared the effects of hypophysectomy by the parapharyngeal route which does not injure the brain with destruction of the gland by the injection of chromic acid which does Only the second operation produced obesity The region responsible for the obesity was later identified by Hetherington and Ranson (1940) who placed bilateral electrolytic lesions in the hypothalamus of rats with an electrode introduced from above to avoid damaging the adjacent pituitary gland Brobeck Tepperman and Long (1943) confirmed this work and localized the region more accurately to the lateral borders of the ventromedial nuclei of the tuber cinereum The way in which hypothalamic damage leads to obesity may be complex Hetherington and Ranson observed both an increase in food intake and a reduction of activity in their animals and regarded them as of equal importance in causing obesity Brobeck and his colleagues were more impressed by the hyperphagia than by the hypomotility and since they made smaller lesions in the brain attributed the difference to this We believe that lesions of this part of the hypo thalamus always affect both appetite and activity (*see* below) and the different emphasis placed on the two effects by previous workers was probably due to the age of the animals they used This is of much greater importance in our experience than the size of the lesion in the brain In general the younger the animal the less

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The direct effect on growth

Children with Frohlich's disease traditionally fail to grow and it is possible to simulate this effect in weanling rats by hypothalamic lesions without direct interference with the pituitary for about one third of the animals upon which we experimented at this age became obese but failed to grow normally (*see* Fig 2)



FIG 2.—Experimental production of lesions of the hypothalamus A comparison of a rat with an unoperated litter mate In spite of its gross obesity the operated rat weighed approximately the same amount as the control rat It had not developed sexually and had some features such as its infantile downy fur which resembled an hypophysectomized animal

Conclusion

This experimental work on Frohlich's disease illustrates the difficulty which Harris (1955) has emphasized of localizing centres in the hypothalamus devoted to the control of single functions Although there is no doubt that the effect of medial hypothalamic lesions may in some circumstances be chiefly on the intake of food it is doubtful whether such an effect can ever be produced in isolation and probable that the centre concerned has multiple co-ordinating functions in the intact animal The suggestion that the animal regulates its output as well as its intake of energy may seem novel but it is in line with recent knowledge of the facilitator and suppressor effects exerted on other parts of the nervous system by the central reticular substance at the head of which the hypothalmus stands (Magoun 1950) In the discussion on the regulation of food intake which follows it is assumed that the hypothalamic centres act in concert with other parts of the nervous system and with the endocrine glands

THE REGULATION OF FOOD INTAKE

General principles

There is no doubt that hunger is aroused by the contractions of the empty stomach and that after eating the distension of the viscus is important in curtailing the intake of food (Cannon and Washburn 1915 Carlson 1916 Janowitz and Hollander 1955) The effect of the latter is labile however and readily modified by the nutritional state of the whole body Small animals like the rat can survive for a much shorter time than man without food and they show an extraordinary capacity to work for calories A young rat will learn within a few days to

striking is the effect of the hypothalamic operation on appetite or on bodyweight (Kennedy 1957a)

Atrophy of the gonads

While working on the relation of diabetes insipidus to the hypothalamus Bailey and Bremer (1921) noted that two of their dogs became obese These were probably the first pair of obese dogs produced in the laboratory They believed that the atrophy of the gonads in these animals was secondary to their obesity Hetherington and Ranson (1942) and Brooks Lambert and Bard (1942) suggested that the two effects were independent since some hypothalamic lesions appeared to cause one without the other This was confirmed by Bruce and Kennedy (1951) who described a variety of disturbances of the oestrus rhythm which occurred in association with hypothalamic obesity but were equally common among rats which failed to become obese through some inaccuracy in placing the lesions One of these effects first described by Dey (1943) in the guinea pig and by Hillarp (1949) in the rat has been called constant oestrus by Harris (1955) since the rats exhibit persistent vaginal cornification It has been suggested that this is the result of interference with the secretion of the luteinizing hormone of the pituitary the follicle stimulating hormone being unaffected In our experience however such animals did not mate or mated infrequently, and their ovaries were small The failure to mate was not necessarily or entirely due to an ovarian defect since it was frequently observed in rats with quite normal ovarian cycles but reduced locomotor activity following hypothalamic damage It seems probable that contiguous areas in the hypothalamus may be concerned with the endocrine and the purely motor aspects of mating

Activity

Spontaneous running activity is usually measured in the laboratory by some form of treadmill Hypothalamic obesity is always accompanied by a great reduction in such activity which is evident immediately after operation before the animal becomes fat Brooks (1946) showed that this was at most a contributory cause of obesity in the adult female rat and further found that the degree of inactivity was not proportional to the degree of obesity The rats he studied however had irregular oestrus cycles and oestrus itself promotes running activity In male rats close correlation between hypomotility and obesity can be demonstrated (Kennedy 1955) although in adults the former is a much less important cause of obesity than hyperphagia the relative importance probably varies with both age and species The findings of Hetherington and Ranson in young rats have been mentioned and Mayer (1955a) has shown that inactivity is a major factor in causing the genetic obesity of mice Johnson Burke and Mayer (1954) found that obese Massachusetts schoolgirls ate no more than their fellows but spent an inordinate amount of time watching television The importance of inactivity in clinical obesity has been minimized most of the books carry statements that unattainable levels of exercise are required to burn insignificant amounts of fat In fact quite moderate exercise such as cycling or brisk walking costs the average man 500 calories an hour (Orr and Leitch 1938 McCance 1953) A daily error of 500 calories in the regulation of energy expenditure if it was a consistent one would lead to the accumulation of 20 kilograms of fat in a year

Post prandial fat synthesis

Mayer (1955) who accepts the evidence that there is a long term regulation of energy stores explains this by the depression of glucose utilization which occurs when stored fat is metabolized Thus the animal could distinguish between freshly absorbed food and reserves and so avoid becoming thin It is more difficult to see how it could avoid becoming fat It is true that the post prandial fat synthesis is associated with increased glucose utilization which at first should produce satiety but this safeguard would become less effective the more it was used Tepperman Brobeck and Long (1943) showed that rats which over ate either because of training or after hypothalamic operation rapidly increased their ability to turn food into fat and consequently used less carbohydrate post prandially Further, Bates Mayer and Nauss (1955) have themselves shown that the fatter an animal becomes the more fat it mobilizes each day and the more carbohydrate it spares Both these effects would ensure that once obesity began it would continue to develop at an ever increasing rate

The ' lipostatic ' theory

The proportion of fat in the body of a young rat remains fairly constant in the absence of hypothalamic damage This might of course simply be a characteristic of growth The disturbance of this steady state of fat stores by hypothalamic lesions however has led Kennedy (1953) to suggest that the central hypothalamus exerts a lipostatic control and that it is not directly concerned with all aspects of appetite regulation but particularly with the long term adjustment of intake to prevent a plethora of fat The rat adjusts its intake of food to the varying demands of activity or environmental temperature which may be considerable so long as sufficient time is allowed for these adjustments Adaptation is just as rapid and efficient after an hypothalamic operation as before Conversely neither the rate of development nor the degree of obesity is affected by such environmentally induced changes in appetite It has therefore been suggested that the real index of the degree of dysfunction produced by an individual hypothalamic lesion is the difference between the new steady state of the fat depots in the so-called static phase of obesity and the pre operative level The disturbance of appetite is very variable and is dependent not only on the factors already mentioned but as we shall show later on the age of the animal The afferent pathway for such regulation could be through the level of some metabolite of fat in the circulation or through the peripheral nerves

Psychological factors

There are obvious differences between the efficiency with which species such as the rat and the pig regulate their energy stores and these may reflect differences in hypothalamic control However that may be it is clear that the hypothalamus is not the only part of the nervous system concerned in the regulation of appetite The effect of cortical lesions in the production of polyphagia has been mentioned and it is probable that in a complicated animal like man psychological factors are of great importance in determining the intake of food In all animals eating is an irregular activity We tend to think nowadays of the satisfaction of hunger in terms of an individual meal of fairly constant size and it is clearly only in this

eat a mixture of three parts of powdered kaolin and only one of its usual diet and so allow the distension of its gut to four times the usual level (Adolph 1947 Kennedy 1950) This drive to maintain calorie requirements under the most unfavourable circumstances and conversely to recognize when sufficient food has been eaten so preventing excessive stores from accumulating has led to a search for some intrinsic regulator of appetite Recent theories have revolved round the chemical changes which take place internally after the absorption of food rather than the mechanical ones which precede and accompany its ingestion Broadly speaking there are two possibilities The animal must either have some mechanism for measuring the heat released from food after its ingestion or it must react to the level of some specific constituent of food in its plasma or tissues

The " thermostatic " theory

This has been favoured by Strominger Brobeck and Cort (1953) who claimed that there was a good correlation between the amount of food eaten in fairly short term experiments and its specific dynamic action Brobeck (1948) summarized this point of view thus animals eat to keep warm and stop eating to prevent hyperthermia This idea is supported by the existence in the hypothalamus of thermosensitive regions adjacent to the part concerned in the regulation of appetite (Ranson 1939) but there are difficulties about accepting it If thermoregulation is the prime consideration diets rich in calories but low in specific dynamic action should cause obesity In fact excessive consumption of such diets by young rats is transient Ingle (1951) showed that the spontaneous tendency to obesity in ageing rats could be exaggerated by feeding a semi fluid high calorie diet and severely restricting activity It is nevertheless difficult to isolate any single cause of such obesity Brobeck s theory also fails to explain how the hypothalamic receptors could distinguish between the heat released in the specific dynamic action of a meal and the far greater amount of heat released during muscular exercise Instead of being interpreted as a signal to eat more the metabolism of exercise should satisfy hunger A purely thermosensitive control could therefore prevent neither obesity nor cachexia Confusion is probably due to the different meanings attached by physiologists to the term calories Although a rat may be said to eat for calories (Adolph 1947) it cannot regulate the amount of potential chemical energy stored in its depot fat by thermal sensitivity

The " glucostatic " theory

The possibility of chemical regulation of appetite has led Mayer (1952) to advance the theory that appetite depends on the rate of glucose utilization by the sensitive cells The actual level of glucose in the blood is not postulated to affect the receptors it may be high as it is in diabetes but the animal remains hungry so long as utiliza tion is small Satiety is achieved only when the blood sugar rises sufficiently to stimulate utilization as indicated by an increased arteriovenous difference in level The weak points of this theory have been discussed by Grossman (1955) Mayer for example has so far been able to measure only the peripheral arteriovenous differences which do not necessarily reflect the metabolic activity of brain cells Nevertheless the finding of Forrsberg and Larrson (1954) that the uptake of ^{3} P by the central hypothalamus differs from that of other parts of the nervous system suggests that this difficulty may eventually be overcome

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context that such factors as gut distension and changes in blood sugar can be con sidered McCance (1953) pointed out that to eat a meal which would today be classed as gargantuan would have represented normal behaviour in earlier times Kennedy (1951) suggested that the overeating in psychogenic obesity is more likely to be the consequence of a disorganization of the feeding pattern in people who are predisposed to obesity by possessing little hypothalamic control than an actual hypothalamic disorder In an essay on the role of the emotions in hunger and appetite Bruch (1955) showed how readily our feeding habits may revert to a more primitive type and how the sudden desire for turkey which afflicts the population of the United States on Thanksgiving Day is a social habit related originally to the fear of famine which haunted the New England settlers She also discusses the effects of starvation after World War II from the point of view of the psycho analyst and in relation to the present prevalence of obesity

OVERNUTRITION

First principles

In order to grow all organisms require an external source of nitrogenous material together with a sufficient supply of calories to enable them to build up and maintain their protein structure Unicellular organisms have a great capacity for growth but if kept in a nitrogen free environment they are unable to exercise it If they contain chlorophyll however these cells can usually obtain more energy by photo synthesis than they require to maintain their protein structure intact and the excess is stored often in the form of fat The same is true of unicellular animals such as Tetrahymena when they are kept in a medium with a high carbohydrate/ protein ratio These are fundamental principles and it has been shown that any thing which limits growth predisposes to obesity Some green algae for example produce chemicals which accumulate in the culture medium and inhibit cell division (Pratt and Fong 1940 von Denffer 1948) it is at this time that they become fat Complex organisms may also produce growth inhibitors (Weiss 1955) and there are many analogies between the development of obesity after growth has been stopped in unicellular organisms and its appearance in adult life in more complicated systems (McCance 1953)

Somatic growth

Increase in the intake of food has not the same effect at every age Parkes (1929) showed that the growth of suckling mice could be greatly accelerated by increasing the supply of milk and we have applied this technique to rats If no more than two or three new born rats are allowed to remain with one mother they grow twice as fast as littermates reared in groups of ten or more The small rats are not thin and the big ones are not fat in fact the composition of the bodies of both groups is remarkably similar When the groups are weaned and allowed free access to the same diet they continue to eat and grow in proportion to their body weight at weaning the bigger rats reach maturity well in advance of the smaller ones This simple experiment shows how difficult it may be to saturate the capacity of a young rat to grow and until this is done there can be no significant deposition of fat During this period of rapid growth a large amount of food is eaten per unit of body

weight and as the animal gets larger this quantity is greatly reduced This reduction is one of the characteristic features of animal growth and if the hypo thalamus of a rat is successfully punctured we believe that the effect whatever the age of the animal is to raise its intake of food per unit of body weight to the level characteristic of the very young animal Thus the effect in an adult is to produce an instantaneous and colossal increase in the intake of food whereas the same operation at weaning time produces an increase which is scarcely measurable at the time but which becomes more and more conspicuous as the weeks go by and the expected fall in the food intake does not take place There are times in the lives of some adult animals when the intake of food may reach the level usual in a very young one this is particularly well demonstrated by the lactating rat As might be expected puncturing the hypothalamus at such a time makes little or no difference to the intake of food

Visceral growth

When the unicellular organism becomes fat the storage material can only be deposited among the working parts of the cell An obese animal however may contain 70 per cent of fat in the body as a whole while its liver contains less than 10 per cent To some extent this is due to the presence in the higher animal of specialized storage organs which can be regarded as protecting the working structures from the effects of overnutrition Some of the active organs notably the liver are also protected from overnutrition by their inherent capacity to grow which they retain after general somatic growth has ceased During the first 2 weeks of hypothalamic hyperphagia the liver of an adult rat doubles in weight without any significant increase in its fat concentration At first this is achieved by an increase in the size of the existing cells but 6 weeks after the operation the number of cells may have increased by 50 per cent Only when the growth of the liver slows down does the amount of fat in the organ begin to increase Thus the adult liver reacts to overfeeding in the same way as the body of the young animal it retains the capacity to react in this way even after hypophysectomy (Kennedy 1957b) Other viscera notably the kidney the heart and the gastro intestinal tract respond similarly to the liver although to a slightly less degree

Growth and obesity in the human child

Man grows much more slowly than the rat and his capacity for growth is relatively easily saturated by an increased intake of food therefore fat but otherwise normal children are quite common whereas fat weanling rats are virtually unknown Wolff (1955) reviewed the evidence that overfeeding accelerated development in the human child and studied 100 obese children who had been within the normal weight range at birth but who had grown faster in height than was to be expected from standard tables derived from a similar social group In both sexes the onset of puberty was approximately 1 year in advance of children of average height The acceleration of development was attributed to overfeeding although the food intake of the children was not measured There is no doubt that growth of the human child as of any young animal is greatly affected by the intake of food Whether the maximum rate of growth is attainable without some measure of obesity is not yet known but it seems probable that the storage of fat indicates that more food is being eaten than can be utilized for normal growth The children Wolff studied

did not grow any faster than non obese children of the professional classes Further the human child is a complex animal and as Widdowson (1951) has shown psychological and environmental factors may greatly modify the rate of growth Meanwhile the reported association between obesity and premature development may be as readily explained on genetic as on nutritional grounds The association of early puberty with increased stature is well established (Stuart 1946) Some fat children have large birth weights (Mossberg 1948) others have not (Wolff 1955) most children big at birth remain taller than those of smaller birth weight (Illingworth 1950) Fat mothers tend to have large children whether they later develop diabetes or not It may well be that some of these effects are due to an abnormally large appetite genetically determined or not and that both the early rapid growth and subsequent obesity are manifestations of this (Widdowson 1955)

Endocrine Aspects of Overnutrition

The influence of hormones on nutrition

Some hormones alter the metabolism of fat or protein and by so doing modify inversely the quantities found in the tissues it is not known how these changes are brought about The nitrogen retention produced by growth hormone is accompanied by the mobilization of fat Levin and Farber (1952) and Greenbaum (1953) have suggested that the primary action of the pituitary growth hormone is the mobilization of fat which provides the energy for the retention of nitrogen Young (1952) favoured the idea that its principle action was the inhibition of protein and carbohydrate catabolism to which the increased metabolism of fat was secondary Since overfeeding in the hypophysectomized rat produces growth without fat in some tissues and fat without growth in others the two processes seem to be as fundamentally opposed in the organs of the higher animals as they are in unicellular organisms The action of hormones in this as in other respects may be to modify existing rather than to initiate fresh chemical reactions

Retention of nitrogen in hypophysectomized animals

Long acting insulins can produce obesity in the rat (Barnes and Keeton 1940) and at first sight this seems to contradict our hypothesis since Salter and Best (1953) showed that insulin causes some retention of nitrogen in the hypophysectomized rat This limited nitrogen retention may be due simply to the stimulation of appetite since the same effect occurs in force feeding (Samuels 1947) or after a hypothalamic puncture in hypophysectomized animals Studies by Beaton and Curry (1956) show that in the intact animal insulin acts inversely on fat and protein retention and in the opposite direction to growth hormone

Cushing's disease

Cushing s disease is characterized by a general loss of protein and an accumulation of fat In the adult the total amount of fat is not great the distribution rather than the amount of the obesity being the cardinal characteristic In the child Cushing s disease is rare and Wilkins (1950) found only 26 cases under ten years of age quoted in the literature all associated with adrenal cortical tumours When it does occur the disease is accompanied by striking obesity cessation of growth

and osteoporosis signs one would expect from a failure of nitrogen retention without a corresponding reduction in appetite and reminiscent of the effects of hypophysectomy with overfeeding

Diabetes mellitus

After growth has ceased the other well established syndrome involving over nutrition and an endocrine abnormality is diabetes mellitus Joslin (1946) stated that the incidence of diabetes is highest where (1) the average age is oldest (2) women predominate (3) obesity is most frequent (4) the proportion of Jews greatest (5) medical supervision closest and (6) deaths most accurately reported Although obesity may sometimes cause diabetes only a small proportion of obese subjects die from diabetes (Thorn 1950) Joslin found nevertheless that 80 per cent of all adult diabetics and 95 per cent of female Jewish diabetics are obese but the genetic problem is complex because neither diabetes nor obesity seem to be inherited as a single genetic entity (Harris 1949 1951 Mayer 1953 Hanssen 1954) Dahlberg (1949) suggested that diabetes is part of a genetic syndrome the first manifestation of which is obesity a similar sequence has been described in mice by Ingalls Dickie and Snell (1950) Mayer (1955b) showed that such mice in the obese prediabetic stage can readily be made diabetic by small doses of growth hormone Apart from such direct genetic linkage obesity can render diabetes more severe or may even cause it in predisposed subjects Thus Brobeck Tepperman and Long (1943) showed that in rats it was possible to reduce the pancreatic reserve by partial pancreatectomy without seriously affecting the glucose tolerance if the animals were of normal weight if the animals were made obese diabetes developed Long (1954) summarized the position by saying

I am greatly impressed by the association between obesity and diabetes The great majority of diabetics are obese prior to the onset of the disease There may be genetic factors in the background but I feel that the core of the problem is the influence of supranormal weight

Obesity and diabetic proneness

Why persons who are too fat get diabetes is not known Lawrence (1951) suggested that when the fat cells had been filled to the limits of their capacity incoming carbohydrate had to circulate in excess but this will not by itself explain why obesity may precede diabetes for many years Ogilvie (1935) showed that glucose tolerance is not impaired in the early stages of obesity while Long (1954) thought that the increased deposition of fat required excessive secretion of insulin which at first stimulated and later exhausted the islet cells of the pancreas Richardson and Young (1937) showed by histological methods and Gaebler and Robinson (1942) and Milman de Moor and Lukens (1951) by indirect chemical means that growth hormone causes a similar sequence of effects on the pancreas of the adult animal Growth hormone diabetes like obesity is very hard to produce in the growing animal and Young (1951) suggested that the hormone may accelerate growth without increasing the tissue demands for insulin Scott and Engel (1950) concluded from studies of the glycogen in adipose tissue that growth hormone did not stimulate insulin secretion in the rat an animal which continues to grow throughout its life If therefore the predisposition to diabetes shown by some individuals is due to increased secretion of growth hormone this might be without

effect on the pancreas during growth but might cause an increased demand for insulin in early adult life and exhaust the islet cells with the onset of obesity in middle age

Production of insulin sensitivity after hypothalamic injury

If an adult animal fails to produce its physiological quota of growth hormone the effect cannot be detected by any change in size but an increase in sensitivity to insulin which can be restored to normal by the administration of growth hormone (Spirtos *et al* 1954) has been demonstrated after hypothalamic injury

UNDERNUTRITION

Yesterday and today

A great deal has been written in the last 10 years about advanced undernutrition (Keys *et al* 1950) Detailed information about famine in the concentration camps has been provided by Lamy Lamotte and Lamotte Barillon (1946) Hottinger *et al* (1948) and Helweg Larsen *et al* (1952) and a Medical Research Council Report (1951) has described the milder undernutrition of a German industrial town In spite of our obesity clinics the threat of famine still hangs over the West (Fenelon 1952 Massingham and Hyams 1953) and near starvation affects many people in less favoured parts of the world today

Simmonds' disease

Different authors mean different things by this diagnosis Sheehan and Summers (1949) pointed out that Simmonds himself did not regard cachexia as a uniform clinical symptom and insisted in all his papers that the essential diagnostic criterion was destruction of the pituitary Sheehan (1939) who reviewed 51 published cases with additional notes from 70 others emphasized that post partum necrosis of the pituitary was the commonest lesion Sheehan and Summers (1949) excluded any case in which there was evidence of damage to the hypothalamus their clinical picture of the condition corresponded to that of surgical hypo physectomy If one accepts this rigid criterion there is little doubt that Simmonds disease and hypophysectomy in the human and hypophysectomy in animals are compatible with an appearance of reasonably good nutrition Most of the patients studied by Sheehan were apparently well nourished until 6 months before their death

Verification of hypothalamic and pituitary lesions

Escamilla and Lisser (1942) based their diagnosis of Simmonds disease on four points (1) loss of weight (2) loss of sexual function (3) asthenia and (4) a very low basal metabolic rate They were able to find 595 cases in the literature in 101 of which there was pathological verification of a hypothalamic or pituitary lesion According to Sheehan and Summers the damage was confined to the pituitary in only 37 of these cases Of Escamilla and Lisser s series 65 per cent were cachectic and in only 42 per cent was the onset post partum

Hypophysectomy and loss of appetite

Hypophysectomy in animals is followed by great diminution in appetite and loss of body weight but since the animal is unable to retain nitrogen in amounts necessary for growth the little food eaten suffices in most cases to maintain reasonable amounts of fat in the body However this is a caricature of normal nutrition The survival of these animals varies with the clemency of the environment (Richter 1937) At moderately low temperatures which cause no inconvenience to intact animals they become thin and die of inanition Conversely force feeding which cannot produce growth makes hypophysectomized animals grow fat more easily than normal animals (Samuels 1947)

Role of the hypothalamus

The role of the hypothalamus in Simmonds disease has been clarified by the work of Anand and Brobeck (1951) who localized in cats and rats a small region in the lateral hypothalamus on the same coronal plane as the area which produces obesity and where lesions produced fatal anorexia The maintenance of a normal energy intake appears to depend largely on a proper balance between these medial and lateral hypothalamic areas and in hypopituitarism only a relatively slight disturbance of this equilibrium is required to make the difference between obesity and cachexia

Anorexia nervosa

There should be no difficulty in distinguishing this syndrome from Simmonds disease for complete destruction of the pituitary results in loss of body hair and secondary sex characteristics Signs of complete endocrine atrophy however are only found with the most severe grade of pituitary destruction and the distinction between anorexia nervosa and the wider diagnosis of Simmonds disease due to causes such as chromophobe adenoma or craniopharyngioma may not be easy Both conditions may show anorexia asthenia gastro intestinal disturbances such as constipation nausea and vomiting or intestinal cramps hypoglycaemia bradycardia and hypotension and a low metabolic rate According to Williams (1950) increased insulin sensitivity and diminished 17 ketosteroid excretion are common in both Simmonds disease and anorexia nervosa although values below 1 milligram every 24 hours are only found in Simmonds disease (normal female 5–17 milligrams every 24 hours male 8–23 milligrams every 24 hours) Anorexia nervosa may be accompanied by signs of endocrine dysfunction which closely mimic all but the most severe grades of pituitary deficiency Since the clinical picture of anorexia nervosa is nowadays thought to result from a compulsively held pattern of behaviour often following some psychic trauma there is no reason why the psychic drive should not be mediated through the hypothalamus and simulate the effects produced by organic lesions of this region It is of particular interest that there seems to be a close connexion between psychogenic obesity and anorexia nervosa and Stunkard (1955) has pointed out how anorexia frequently begins during an ill advised course of dietary restriction especially in a young obese person

Endocrine relationships of primary undernutrition

Effect on the gonads

There is no doubt that undernutrition interferes with reproductive function almost all reports on interned prisoners agree about this There is disagreement however on the mechanism of production Bass (1947) Stroink (1947) and Dean (1949) found that the onset of amenorrhoea in women followed so quickly upon imprisonment that they concluded that the cause was psychic In one of the most complete studies available Sydenham (1946) concluded that although emotional shock and environmental change undoubtedly explained the initial amenorrhoea the long continued cases were probably nutritional

Report on Rotterdam —There was a serious deterioration in food supplies in Rotterdam in September 1944 which continued throughout the first half of 1945 Good vital statistics are available which show that a sharp decline in birth rate among the civilian population began in June 1945 (Smith 1947) There are many other reports of precipitous declines in birth rate in civilian populations during war but the Rotterdam report is the only one in which complicating factors such as call up of the younger men or failure of collection of statistics can be excluded There are a number of reports of delayed onset of puberty in undernourished children but this may be explained by the general retardation of growth and development in undernutrition (Talbot and Sobel 1947)

Atrophy of the human generative organs —Extreme undernutrition in adults may produce atrophy of the generative organs Butler is quoted by Keys *et al* (1950) as saying that this was sometimes so extreme in Japanese camps that close inspection was necessary to tell the sexes apart This seems hard to believe for moderate undernutrition which produces considerable loss of body weight may have no effect on the genitalia (Klatskin Salter and Humm 1947)

In animals ovarian atrophy uterine atrophy and anoestrous vaginal smears have been reported by numerous authors following inanition There is evidence that this is due to interference with the release of gonadotrophins by the pituitary since normal ovarian and uterine weight can be restored by injecting these sub stances (Marrian and Parkes 1929) Assay of the gonadotrophin *content* of the pituitary in chronic inanition has shown it to be normal The gland is somewhat reduced in size but the potency per gramme of pituitary tissue actually increases It seems therefore that the impact of undernutrition is upon the release and not upon the production of gonadotrophic hormone (Ershoff 1952) This suggests that the hold up is mediated through the hypothalamus

Harris (1955) has drawn attention to the close relationship in animals between the effects of light and food Both the duration of light and the diet affect the onset of oestrus in some animals Further Alexander and Frazer (1952) have shown that the effect of inadequate diet may to some extent be compensated by extra lighting or vice versa The effect of light on oestrus has been extensively studied (Bissonette 1932 1938 Clark McKeown and Zuckerman 1939) and is almost certainly mediated by the hypothalamus It seems likely that both the immediate ' psychic effect observed in war prisoners and the response to more prolonged undernutrition may be mediated through the same final common path

Effect on thyroid activity

Basal metabolic rate—In a well nourished person overactivity of the thyroid gland raises the basal metabolic rate and expenditure of energy This is not as a rule compensated for by a sufficient increase in the consumption of food and so thyrotoxicosis is usually accompanied by wasting and undernutrition Primary undernutrition however does not lead to hypertrophy of the gland just the reverse in fact all accounts agree that this organ atrophies in famine victims and it is particularly marked in infantile athrepsia (Marfan 1921) Brull (1945) reported that the average basal metabolic rate of hyperthyroid patients attending his clinic fell by 15 per cent during the German occupation of Belgium This has found agreement in other accounts

Appearances simulating myxoedema—During acute malnutrition in the Philippines appearances simulating myxoedema were frequently seen (Gottlieb 1946) This atrophy of the thyroid seems to be brought about in a way similar to that of the gonads since in undernourished guinea pigs normal thyroid structure can be restored by thyrotrophic hormone (Stephens 1940) A particularly interesting feature of these experiments is the increase in sensitivity of the thyroid of the undernourished animals to the stimulating hormone it may be 10 times the normal A similar increase in sensitivity has been found in hypophysectomized animals D Angelo (1951) reported decreased levels of thyroid stimulating hormone in the circulation of starved animals and as the thyroid gland is less responsive to goitrogens it has a reduced uptake of ^{131}I (Meites and Agrawala 1949)

Thyrotrophic and gonadotrophic secretion—Clinicians have often suggested that the thyroid secretion might be under the control of the hypothalamus because of the association of psychic trauma with the onset of Graves disease (Thompson 1948) According to Ganong Frederickson and Hume (1954) lesions in the hypothalamus of dogs may reduce the thyroid uptake of ^{131}I to the level found after hypophysectomy The site was slightly farther forward in the median eminence than the region concerned in the control of energy balance and was in the general area which appears to control gonadotrophin secretion It is possible however to alter the secretion of thyrotrophin and gonadotrophin independently (Bogdanove and Halmi 1953 Ganong Frederickson and Hume (1954) Hypothalamic stimulation has not produced unequivocal results and section of the pituitary stalk according to most workers reduces but does not abolish thyroid activity (Harris 1955) Harris suggested that the pituitary secretion of thyrotrophic hormone is decreased by most forms of environmental stress but that this effect has not yet been proved to be mediated always through the hypothalamus

Diabetes mellitus

The onset and the severity of diabetes are both unquestionably affected by the state of nutrition The general effects of undernutrition are the converse of those of overnutrition and these have already been discussed Until insulin was discovered the treatment of the disease was in fact based on undernutrition and remained so for many years afterwards In both the world wars the experience of physicians among semi starving communities was that diabetes became a relatively mild and unimportant disease However the true relationship between the cause or causes and the effects has not yet been scientifically proved

Corticotrophin and the general adaptation syndrome

During starvation both in man and in animals changes in adrenal weight and structure have been reported by many observers Unlike the agreement on the gonads and the thyroid however different observers are far from agreement as to whether the adrenal enlarges or atrophies While abnormally large and abnormally small adrenals have undoubtedly both been observed in starved human beings Schilf (1922) in a series of more than 2 000 autopsies could find no evidence that nutrition produced any consistent change in adrenal weight The observation of Lamy Lamotte and Lamotte Barillon (1948) that the adrenals of prisoners who died soon after liberation from internment were depleted of lipids whilst in the adrenals of those who survived for some months there was a progressive increase suggests that the effect of starvation on the human adrenal may be on function rather than on size In animals it seems clear that the effect of starvation depends on its duration acute starvation produces large hyperaemic and friable adrenals (Jackson 1915 McCarrison 1919 Mulinos and Pomerantz 1941 Cameron and Carmichael 1946) Chronic undernutrition on the other hand is reported by some authors to be associated with adrenal atrophy (Mulinos and Pomerantz *1941*) whilst others have found no adrenal change (Cameron and Carmichael 1946)

Two interpretations of these findings are current Selye (1951) has indicated the importance he attaches to undernutrition as a cause of his general adaptation syndrome On this hypothesis shortage of food is one of the many environmental factors which act through the central nervous system and anterior pituitary gland to produce a sequence of changes in adrenal function with an early phase of over activity eventually succeeded by a stage of exhaustion On the second hypothesis *of Mulinos and Pomerantz (1940 1941) undernutrition produces a condition of* pseudo hypophysectomy

Adrenal atrophy—The adrenal atrophy in chronically underfed rats reported by these authors was not confirmed by other workers and Ershoff (1952) suggested that it may have been due to an unbalanced diet rather than to a protein or calorie deficiency Selye (1945) has shown that the adrenal glands atrophied during under nutrition on a low protein high carbohydrate diet but not on a diet of more normal composition Species differences in animals also affect the adrenal response to starvation It is to be noted that systemic stress in any form may alter the requirements of the body for many specific dietary constituents (Ershoff 1952) and this may explain many of the discrepancies in observations on famine victims

Secretion regulation of adrenocorticotrophin hormone—There are a number of theories on the regulation of the secretion of ACTH from the pituitary gland which is obviously of central importance in the adaptation syndrome These are fully discussed by Harris (1955) whose general conclusion is that the maintenance of normal ACTH secretion under quiescent conditions is almost entirely due to stimuli originating in the hypothalamus whilst increased secretion during stress may be produced either by an increase in these or by a direct pituitary response to changes in the circulating blood The part of the hypothalamus concern d in this control has not been definitely identified but there is no doubt that it is in the posterior region and either in the mammillary bodies or in the area between them and the pituitary stalk (de Groot and Harris 1950 Brobeck 1952 Hume 1953 and Porter 1953)

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CHAPTER 8

THE PITUITARY ANTIDIURETIC HORMONE (ADH) AND THE CONTROL OF FLUID BALANCE

D A K BLACK

THERE is ample evidence that the pituitary antidiuretic hormone (ADH) is one of the continuous regulators of fluid balance and that its absence produces dramatic clinical effects—the syndrome of diabetes insipidus

ADH AND NORMAL FLUID BALANCE

The main *stimulus* to ADH is an increase in the effective osmotic pressure of the plasma and the main *effect* of ADH is to increase the reabsorption of water in the renal tubules In this way a scanty urine of high specific gravity and solute content is produced the reabsorption of water lowers the osmolarity of the plasma and so inhibits further production of ADH This sequence has been stated summarily in order to emphasize at the outset the exquisite appropriateness of response to stimulus this in cybernetic terms is a good example of negative feed back in the regulation of a biological steady state

Posterior pituitary extract already known to have a vasopressor action in high dosage was found to have an antidiuretic action by Magnus and Schafer (1901) *The physiological relevance of this action was first clearly shown by Starling and* Verney (1925) who found that the spontaneous water diuresis of the isolated kidney was controllable by small amounts of posterior pituitary extract It was again Verney (1947) who established the significance of plasma osmolarity as the stimulus to ADH release He showed that the receptors of this stimulus were not in the pituitary itself but elswhere in the distribution of the internal carotid artery and very probably in the supra optic nuclei (Verney 1954) These notable advances in knowledge of ADH secretion as a mechanism of homeostasis have been accom panied by equally brilliant work on the anatomical definition of the structures involved (Fisher *et al* 1938) which will be outlined in relation to diabetes insipidus and by the studies which led du Vigneaud (1954) to the chemical identification and synthesis of ADH now known through his work to be a polypeptide of eight amino acids

Production of ADH

Effect of isotonic and hypertonic saline solution

The critical experiments in defining the *stimulus* to ADH production are those of Verney (1947) and were carried out on well hydrated dogs with a carotid loop into which injections could be made without disturbance to the animal Control injections into the loop of isotonic saline solution and injections of small amounts

of hypertonic saline solution into a vein were without effect on the flow of urine Injection of hypertonic saline solution into the carotid loop produced a suppression of water diuresis similar to that induced by intravenous injection of ADH this response to hypertonic saline solution was abolished by ablation of the posterior lobe of the pituitary That it was a response to increased osmolarity of the plasma and not to sodium chloride specifically was shown by an equal response to hyper tonic sodium sulphate and by a somewhat smaller response to hypertonic dextrose With hypertonic urea on the other hand no response was obtained indicating that for an increase in osmolarity to be effective in this context it must be caused by particles which do not readily enter cells In the light of these experiments, effective osmotic pressure of the plasma can be defined as the osmotic pressure due to those electrolytes notably sodium and its accompanying anions which do not rapidly enter cells

Osmotic pressure so defined accounts for over 90 per cent of the total osmotic pressure of plasma and it has long been recognized as one of the more jealously guarded components of that fixity of the internal medium which Claude Bernard divined to be the prerequisite of life under changing external conditions The high sensitivity of this mechanism to its appropriate stimulus is clearly shown by the observations of Verney (1954) that an increase of only 2 per cent in the osmolarity of the plasma perfusing one common carotid artery constitutes an adequate stimulus to ADH secretion This response to a 2 per cent change implies a much more sensitive regulation of osmotic concentration than of such things as the total sodium or potassium content of the body or the volume of plasma

Transmission of stimulus

There is some circumstantial evidence (Verney 1954) that this osmotic stimulus acts primarily on specialized structures in the supra optic nuclei of the hypothalamus —the osmoreceptors —which then stimulate the posterior lobe to release ADH into the circulation The mode of transmission of the command to secrete ADH from the hypothalamus to the posterior pituitary is not certain section of the pituitary stalk is known to interrupt it but this could be by a block either of neural or hormonal transmission in the pituitary portal venous system (Green and Harris 1947) The site of the actual formation of ADH is hypothalamic but it is stored in the hypophysis The final stage in transmission is through the general circulation to the kidney and during antidiuresis of pituitary origin ADH is demonstrable in blood and urine the amount of circulating ADH necessary to act on the kidneys is very small of the order of 0 00001 milligram (Goodman and Gilman 1955) Circulating ADH is destroyed in less than 5 minutes so the delay of some 20 minutes in the diuretic response to ingested water is largely due to the time needed for water absorption

Renal action of ADH

Increase in water reabsorption

Although the vasopressor action of ADH may be of some consequence in determining antidiuresis in the amphibia it has no significant effect on glomerular filtration rate in mammals it exerts its action by increasing tubular reabsorption

of water In man about 120 millilitres of water is filtered each minute and the amount in the urine usually ranges from 0 5 to 10 millilitres per minute at the height of water diuresis and in diabetes insipidus the urine volume may rise to 20 millilitres per minute It is clear from this that only a part about 15 per cent of the total water reabsorption in the tubules is under ADH control and this has been termed the facultative reabsorption of water (Smith 1951) The obligatory reabsorption of water probably takes place in the proximal tubule and in the loop of Henle while facultative reabsorption occurs in the distal convoluted tubule and possibly in the collecting tubules There is now evidence that the kidneys can form a hypertonic urine by a process independent of ADH Berliner and Davidson (1956) have shown that constriction of one renal artery in the water loaded dog may induce hypertonic urine formation on that side while the other kidney continues to excrete a dilute urine Wirz (1956) has obtained some evidence that ADH may act by allowing water to traverse the wall of the distal tubule so that the fluid entering the collecting tubules is isotonic and may then be concentrated further by a mechanism independent of ADH Increase in water reabsorption is certainly the outstanding effect of ADH on renal function An increase in the output of sodium and chloride would seem to be as reasonable a response to increase in plasma osmolarity as is diminished water output and a natriuretic or chloruretic action of ADH has often been described Much of the work supporting this effect concerned short term changes in sodium or chloride output though this type of experiment is easily falsified by diurnal variation in electrolyte output or even by a simple confusion between sodium output in unit time and sodium concentration which naturally rises as the urine output falls In comparing successive 24-hour periods with and without pitressin tannate Black and Thomson (1951) found no evidence of an increase in sodium or chloride output

Major effect of ADH on fluid balance

The simple picture which emerges is of an increase in plasma osmolarity leading to ADH release which in turn causes increased renal reabsorption of water with a consequent dilution of plasma conversely water drinking lowers the osmotic pressure of plasma decreasing or abolishing ADH output and so decreasing water reabsorption While this statement summarizes the major effect of ADH on fluid balance a few divergent points of detail must be noticed

Control of osmolarity—Although the ADH mechanism is closely implicated in the control of osmolarity it does not stand alone being complemented by the thirst mechanism to which hypertonic saline solution injection is an effective stimulus (Wolf 1950) It is of considerable interest that a thirst centre has been found in the goat in close anatomical relation to the hypothalamic osmoreceptors of the ADH mechanism (Andersson 1956)

Release of ADH—There is a good deal of evidence summarized by Heller (1956) that decrease in the volume of extracellular fluid or of plasma may constitute an adequate stimulus to ADH secretion in the absence of any increase in plasma osmolarity Release of ADH occurs also in response to emotion pain anaesthesia and various drugs such as morphine and nicotine These may all be regarded as exceptional responses which do not displace the increase in effective plasma osmolarity from its primary position as the main physiological stimulus to ADH output

Direct action—The *direct* action of ADH on renal performance in man is limited to an enhancement of water reabsorption and does not significantly affect electrolyte

output there is however no doubt that the sustained action of administered ADH in over hydrated subjects is associated with an increase in sodium excretion (Leaf *et al* 1953) There is now further evidence that this is a secondary effect of expanded body fluid volume which is probably mediated by suppression of aldosterone secretion (Wrong 1956)

Even with these reservations in mind it seems permissible to regard the ADH mechanism as one which is primarily stimulated by and responsible for the regulation of changes in the effective *osmolarity* of plasma The relation of the ADH mechanism to control of the *volume* of body fluid is secondary and indirect the main regulation of this parameter must be sought elsewhere probably in the adrenal cortex

ADH AND ABNORMAL FLUID BALANCE

A primary and permanent over production of ADH has not been described deficiency of ADH production is the basis of the syndrome of diabetes insipidus (*see below*) Considered here is the part played by ADH in those disturbances of fluid balance which arise from general causes and in which the ADH mechanism is not intrinsically deranged so that it is in a position to respond in proper fashion to its normal stimuli The ADH mechanism is involved to some degree in every change in fluid balance and demonstrably so when the plasma sodium concentration is increased or diminished but only two situations need be considered in each of which considerable emphasis has been placed on ADH disturbance as a causal factor

Post-operative oliguria

Very little urine is formed in the first 24 hours after a major operation and some degree of oliguria may persist for several days The urine formed is concentrated and of high specific gravity resembling that formed under ADH influence an increased output of ADH in the urine has been demonstrated by Cline Cole and Holden (1953) Although there seems little doubt that ADH is closely concerned with oliguria in the immediate post operative period other factors such as diminished circulation to the kidneys and an increased formation of salt retaining adrenal steroids are also of importance The release of ADH is probably related to a combination of anaesthesia morphine and post operative pain and the variable duration of the ADH effect may be related to the varying time for which pain persists and needs relief by morphine The practical importance of ADH release after operation is that it prevents the prompt excretion of administered water and so makes the patient vulnerable to water intoxication (Wynn and Rob 1954)

Generalized oedema

The relative importance of ADH in states of generalized oedema is probably much smaller than in post operative oliguria Admittedly increased urinary output of substances which induce oliguria in test animals has often been detected in cardiac renal and hepatic oedema but Van Dyke (1953) has adduced convincing evidence that these antidiuretic substances are distinct from pituitary ADH Oedema depends on a retention of sodium as well as of water and there is no

evidence that ADH can induce sodium retention in so far as ADH action leads to expansion of body fluid it promotes sodium excretion Given a primary cause of sodium retention (and excess of aldosterone seems to be intimately concerned in this) the subsequent retention of water to prevent hypernatraemia is likely to be achieved in part through the ADH mechanism and in part by an increased intake It is difficult to believe that ADH action is maintained when oedema has become established Not only is there often hyponatraemia and always expanded body fluid (conditions the reverse of those known to cause ADH release) but there is no diuretic response to ethanol which is an effective inhibitor of ADH release (Lamdin *et al* 1956) The possibility remains that ADH already formed is inactivated more slowly than normal but apart from phases of active water retention as oedema fluid rapidly increases there seems no real need to invoke the ADH mechanism and there is some evidence against its continued operation ADH may indeed have a more significant part in those patients who have oedema associated with a low concentration of sodium in the plasma—the low salt syndrome A preliminary report by Murdaugh (1956) indicates that such patients unlike those studied by Lamdin *et al* (1956) might show a striking diuretic response to ethanol suggesting that in them ADH was continuously operating

DIABETES INSIPIDUS

The physiological basis of diabetes insipidus has been outlined in describing the origin of ADH The anatomical basis was established by Fisher Ingram and Ransom (1938) who carried out experiments on cats and monkeys using either electrolytic lesions in the hypothalamus produced by the Horsley Clarke stereotaxic instrument or surgical removal of defined portions of the pituitary glands They summarized their findings on the hypothalamic lesions as follows Our analysis of the anatomical data seems to indicate that the only lesions which are capable of producing a diabetes insipidus are those which interrupt bilaterally the supraoptico hypophyseal tracts and result in atrophy of the supraoptic nuclei and the neural division of the hypophysis Destruction of the posterior hypothalamus and of the tubero hypophyseal tract which arises from it does not cause any apparent dis turbance of water metabolism

With regard to lesions of the pituitary gland itself they confirmed previous work showing that severe damage to the anterior pituitary would prevent the occurrence of diabetes insipidus after ablation of the posterior pituitary they found that thyroid extract would not annul this effect of anterior lobe damage (Later work has shown that this failure of water diuresis in anterior lobe hypopituitarism is probably mediated through the adrenals and that it can be corrected by giving cortisone)

The same authors also found that removal of the pars nervosa even with an intact anterior lobe did not in itself cure diabetes insipidus Experimental diabetes insipidus followed removal of the pars nervosa providing that the infundibular stem and median eminence were simultaneously removed or damaged Likewise this disorder developed when the infundibular stem and median eminence were removed leaving the pars nervosa behind This finding supports the view that ADH is actually formed in the hypothalamus and that the posterior pituitary itself is merely a storage organ for ADH

Aetiology

The lesions which can produce in man the anatomical deviation needed to induce diabetes insipidus are varied in their pathology They include tumours active or quiescent inflammatory disease granulomas infarction the effects of trauma and rare causes such as the reticuloses actinomycosis and pellagra (Soffer 1956) In an analysis of 160 cases (Fink 1928) tumour of the hypothalamus pituitary or posterior fossa structures accounted for just over half while half of the remainder were inflammatory usually postencephalitic or related to basal meningitis vascular damage (8) and trauma (9) were found in 17 cases This analysis based as it is on autopsy results probably underestimates the importance of trauma at least as a cause of temporary diabetes insipidus which is not uncommon after head injury and usually clears up in a few weeks A hereditary syndrome of diabetes insipidus is well established (Forssman 1945) but some of these patients do not respond to ADH and have therefore been described as nephrogenic diabetes insipidus (Williams and Henry 1947) The syndrome which is uncommon affects both male and female and is more frequent in children than in adults

Clinical features

The cardinal symptoms are polyuria and thirst the daily turnover of fluid being usually of the order of 15–20 litres though a patient of Trousseau s has earned his place in many text books by excreting 43 litres of urine in a day There has been much argument as to the primacy of thirst or polyuria (Wolf 1950) and there seems little practical advantage in taking sides in this dispute while our concepts of ADH action would suggest that polyuria precedes and indeed leads to thirst the finding of a thirst centre in the hypothalamus makes us hesitate to exclude a primary polydipsia in every instance Most patients with diabetes insipidus show some general symptoms of water depletion which improve when the polyuria is arrested by ADH—this is in support of the polyuria being primary The urine formed in diabetes insipidus is water pale and of very low specific gravity and concentration nor can these characters be greatly modified by fluid restriction unless this is pushed much against the patient s wishes to gross dehydration when the specific gravity may rise to 1010 By contrast ADH injection allows the formation of a concentrated urine

Compatibility with normal health

Although the polydipsia and polyuria interfere greatly with a normal life persisting as they do through the night and without regard to occupation they are nevertheless compatible with many years of otherwise normal health

Mrs A H aged 45 years had been subject to thirst and polyuria since the age of 13 years the onset was sudden and unrelated to any illness which she can recall She was drinking several glasses of water each hour both day and night and had to take several bottles of water when she went to the cinema Medical advice was not sought until the development of a cystocele caused her discomfort On admission to hospital she was found to be a nervous woman and was thought to be a compulsive water drinker She would not allow simple restriction of her fluid intake which ranged between 15 and 20 pints a day with corresponding polyuria A pint of 5 per cent saline solution was given intravenously on two occasions with no appreciable effect

evidence that ADH can induce sodium retention in so far as ADH action leads to expansion of body fluid it promotes sodium excretion Given a primary cause of sodium retention (and excess of aldosterone seems to be intimately concerned in this) the subsequent retention of water to prevent hypernatraemia is likely to be achieved in part through the ADH mechanism and in part by an increased intake It is difficult to believe that ADH action is maintained when oedema has become established Not only is there often hyponatraemia and always expanded body fluid (conditions the reverse of those known to cause ADH release) but there is no diuretic response to ethanol which is an effective inhibitor of ADH release (Lamdin *et al* 1956) The possibility remains that ADH already formed is inactivated more slowly than normal but apart from phases of active water retention as oedema fluid rapidly increases there seems no real need to invoke the ADH mechanism and there is some evidence against its continued operation ✓ADH may indeed have a more significant part in those patients who have oedema associated with a low concentration of sodium in the plasma—the low salt syndrome A preliminary report by Murdaugh (1956) indicates that such patients unlike those studied by Lamdin *et al* (1956) might show a striking diuretic response to ethanol suggesting that in them ADH was continuously operating

DIABETES INSIPIDUS

The physiological basis of diabetes insipidus has been outlined in describing the origin of ADH The anatomical basis was established by Fisher Ingram and Ransom (1938) who carried out experiments on cats and monkeys using either electrolytic lesions in the hypothalamus produced by the Horsley Clarke stereotaxic instrument or surgical removal of defined portions of the pituitary glands They summarized their findings on the hypothalamic lesions as follows Our analysis of the anatomical data seems to indicate that the only lesions which are capable of producing a diabetes insipidus are those which interrupt bilaterally the supraoptico hypophyseal tracts and result in atrophy of the supraoptic nuclei and the neural division of the hypophysis Destruction of the posterior hypothalamus and of the tubero hypophyseal tract which arises from it does not cause any apparent dis turbance of water metabolism

With regard to lesions of the pituitary gland itself they confirmed previous work showing that severe damage to the anterior pituitary would prevent the occurrence of diabetes insipidus after ablation of the posterior pituitary they found that thyroid extract would not annul this effect of anterior lobe damage (Later work has shown that this failure of water diuresis in anterior lobe hypopituitarism is probably mediated through the adrenals and that it can be corrected by giving cortisone)

The same authors also found that removal of the pars nervosa even with an intact anterior lobe did not in itself cure diabetes insipidus Experimental diabetes insipidus followed removal of the pars nervosa providing that the infundibular stem and median eminence were simultaneously removed or damaged Likewise this disorder developed when the infundibular stem and median eminence were removed leaving the pars nervosa behind This finding supports the view that ADH is actually formed in the hypothalamus and that the posterior pituitary itself is merely a storage organ for ADH

water experimentally find it difficult to resume a normal intake they feel thirsty while doing so One possible explanation for this has been discovered by de Wardener (1956) He has shown that such patients when allowed to drink freely and given Pitressin and 25 per cent mannitol cannot concentrate their urine after 12–24 hours of fluid restriction the concentrating power of the kidneys improves but still not up to normal levels One patient after a week of fluid restriction produced an almost normal urine concentration with Pitressin and mannitol There was a lack of response to nicotine in high doses These findings suggest that a prolonged high intake of water prevents the kidneys from responding normally to Pitressin and that this relative unresponsiveness may take some time to wear off

Nephrogenic diabetes insipidus

Large losses of water in a urine of very low specific gravity have been observed in advanced renal disease (Roussak and Oleesky 1954) and also in the condition described as nephrogenic diabetes insipidus The mechanism of so-called nephrogenic diabetes insipidus is not a lack of ADH as in true diabetes insipidus but a failure of response to ADH on the part of the appropriate end organ the renal tubule

Distinction between true and spurious diabetes insipidus

The distinction between true diabetes insipidus and the simulating states of psychogenic polydipsia and primary renal water loss is theoretically simple with the aid of the response to water deprivation to ADH and to hypertonic saline solution Primary renal water loss is resistant to Pitressin the other conditions respond Patients with true diabetes insipidus and renal water loss continue to pass large volumes of urine even when deprived of water intake Patients with psychological polydipsia show an antidiuretic response to hypertonic saline solution this is absent in diabetes insipidus and in renal water loss In practice however these tests may present serious difficulties in performance or in inter pretation

Water deprivation —This is very poorly tolerated by patients with either diabetes insipidus or renal water loss for their polyuria continues until their water deficit is so great as to interfere with the circulation to the kidneys Patients with psycho genic polydipsia might in theory be expected to respond to water deprivation with a fall in urine output but the work of de Wardener (1956) indicates that their response while better than that in diabetes insipidus still falls far short of the normal moreover thirst persists so that close observation is required to prevent evasion of the water restriction Patients with diabetes insipidus may quite properly refuse their consent to any adequate period of water deprivation For these various reasons the test is not of great value and is not free from risk in diabetes insipidus and renal water loss

ADH test doses —The use of a test dose of ADH is of considerable value in separating off the renal water losers The ADH is given intravenously as an aqueous solution of Pitressin and a dose of the order of 0 1 unit should be used Much smaller doses are of course effective in producing antidiuresis in normal subjects and in diabetes insipidus patients with psychological polydipsia are less responsive In addition to measuring the volume of urine produced the

on urine volume specific gravity (1002 throughout) or chloride concentration (2-5 milliequivalents per litre) She responded promptly to posterior pituitary extract with a fall in urine volume increase in specific gravity and alleviation of thirst her water turnover fell to 5 pints per day and her cystocele was relieved by operation In spite of this very long history of polydipsia and polyuria her serum electrolyte concentration was normal (Na 142 mEq /l K 4 7 Cl 102 and HCO_3 28) she had no clinical evidence of sodium depletion and her blood urea was normal (32 milligrams per 100 millilitres)

Latent effects

The pure syndrome of diabetes insipidus can be both modified and complicated by the effects of the underlying lesion When this spreads to involve the anterior pituitary, the polyuria and polydipsia disappear A variety of local symptoms may be added to the diabetes insipidus the most important being headache visual disturbance, and change in appetite or temperature regulation Polyuria and polydipsia may, however precede any demonstrable local effects by some years as exemplified in the following case report

C M a schoolgirl was first seen in November 1951 at the age of 9 years with an 18 month history of increasing polyuria and thirst In January 1952 her maximum urine specific gravity after overnight water depletion was found to be 1005 ADH was given and produced a concentrated urine and abolished the polyuria At that time the visual fields were full and a radiograph of the skull was normal She was given maintenance treatment with Pitressin Tannate at first she needed 2 5 units twice weekly She was kept under observation and remained well in herself until early in 1955 when she complained for the first time of headache and some drowsiness Her parents had noted a gradual decrease in her requirement of Pitressin Tannate and by May 1955 she needed only 2 5 units per week On examination optic atrophy and visual impairment were found Investigation in the neurosurgical department showed evidence of a tumour involving the hypothalamus and the third ventricle associated with a hydrocephalus She no longer had thirst or polyuria but required a small dose of cortisone for anterior lobe insufficiency

Differential diagnosis

The aetiological diagnosis of diabetes insipidus is a problem for neurological and neurosurgical investigation and here we can deal only with the means of distinguishing true diabetes insipidus from other conditions which may simulate it General causes of polyuria such as diabetes mellitus and renal failure are not likely to cause much difficulty since the urine volume rarely exceeds 5 litres per day and direct evidence of these conditions can usually be found from history examination urinalysis and on occasion an estimation of the blood sugar or urea Amounts of urine quite comparable to those passed in diabetes insipidus may be observed in patients who have hysterical polydipsia (compulsive water drinkers) and also in a few patients with renal disease who have specifically lost the ability to concentrate the urine even to a specific gravity of 1010

Psychological disturbance

A number of patients with psychological disturbance acquire the habit of water drinking sometimes in fantastic amounts and quite comparable to those taken by patients with diabetes insipidus Once established this condition is difficult to throw off and even normal people who have taken large amounts of

by the intramuscular injection of Pitressin Tannate in oil (Thorn and Stein 1941) The defect in diabetes insipidus is often incomplete so that the frequency of injections needed to control symptoms has to be determined by trial in each patient With injections of 5 units of Pitressin Tannate suspended in 1 millilitre of arachis oil many patients can be controlled by injection on alternate days and some by bi weekly injections Patients can be trained to give their own injections they must be told to warm the ampoule to body temperature and to shake it very thoroughly before transfer to the syringe as the activity is entirely in the grey white powder which sinks to the bottom of the ampoule A few patients develop allergic reactions to the Pitressin powder but this is rare even with prolonged treatment some patients may be able to control their polyuria sufficiently with posterior pituitary snuff, given in 40–50 milligram doses by nasal insufflation and at intervals of 6–10 hours

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chloride concentration should be measured In their two patients with renal water loss Roussak and Oleesky (1954) found a slight fall in urine volume but no increase in chloride concentration In conformity with their lack of response to administered ADH patients with renal water loss have been found to have anti diuretic activity in their plasma which is absent in diabetes insipidus but the difficulties of assay prevent the estimation of antidiuretic activity in plasma from being a practical clinical test

Hypertonic saline solution test —The most useful test for distinguishing diabetes insipidus from psychological polydipsia is the hypertonic saline solution test of Hickey and Hare (1944) as modified by Cates and Garrod (1951) After a preliminary period of water deprivation if this is tolerable a water diuresis is induced by drinking 20 millilitres of water per kilogram body weight within an hour After a further hour during which the bladder may be catheterized an infusion of 2 5 per cent saline solution is given for 45 minutes at a rate of 0 25 millilitres per kilogram per minute In normal people this procedure induces a striking fall in the previously high urine volume and indicates integrity both of osmotic responsiveness by the osmoreceptors and of the subsequent formation of ADH and its action on the kidneys In true diabetes insipidus this response is absent and the urine volume actually rises in consequence of the saline induced osmotic diuresis In one patient with functional polydipsia Cates and Garrod (1951) found a normal response on two occasions to the hypertonic saline solution test and Hickey and Hare (1944) reported similarly of their test

Nicotine test —A patient who responds to administered Pitressin but who fails to produce an antidiuresis after hypertonic saline solution and who has no overt disorder of personality is very likely to have true diabetes insipidus for which a cause should be sought Some help in the localization of the defect may be obtained from the nicotine test (Lewis and Chalmers 1951 Cates and Garrod 1951) This test depends on the fact that nicotine like acetylcholine stimulates the release of ADH independently of the osmoreceptors The combination of an antidiuretic response to nicotine and no response to hypertonic saline solution therefore suggests a lesion involving the osmoreceptors themselves and which does not directly involve the pituitary The nicotine test has the additional merit that it can be carried out in patients who are unfit to withstand the very considerable expansion of plasma volume induced by the hypertonic saline solution test (Lewis and Chalmers 1951) These workers found that the results of the nicotine test alone gave a clear difference between 7 patients with diabetes insipidus and 3 patients with psycho genic polydipsia The test can be carried out in previously hydrated subjects either by inhaling deeply the smoke of 1–3 cigarettes until nausea is produced (Lewis and Chalmers 1951) or by giving intravenously 1–2 milligrams of nicotine to non smokers and up to 6 milligrams to smokers (Cates and Garrod 1951)

Treatment

Since the output of urine depends not only on water reabsorption but also on the amount of urea and salt being excreted the polyuria of diabetes insipidus can be abated to some extent by a low protein low salt diet This is merely a palliative measure and by no means replaces ADH substitution treatment Aqueous pituitary extract has an action too short to be of practical value Dried pituitary extract in the form of snuff has been used but the most effective maintenance treatment is

as hydrocortisone especially if sodium loads are previously administered to the rat Positive results are however given in the test of Simpson and Tait (1952) in which decrease of the sodium potassium ratio is measured in the urine of rats which have not received a sodium load but enhancement of potassium excretion is in part associated with protein catabolism and possibly not entirely due to direct effects on the kidney associated with electrolyte balance Nevertheless the tests give parallel results Although a defect in electrolyte metabolism is probably not the direct cause of death in adrenalectomized animals the ability to maintain life by different corticoids in the adrenalectomized dog is strikingly parallel to their sodium retaining effects Owing to difficulties from loss of dogs with minimal doses for life maintenance control of the blood non protein nitrogen level is now used and is an excellent parallel method of assay (Table I Pfiffner Swingle and Vars 1934)

Tests measuring the survival of adrenalectomized rats are complicated by a further factor in that ability to maintain growth in these animals is also used as a criterion of activity (Cartland and Kuizenga 1936) A further steroidal property is therefore measured and doubtless properties associated with the regulation of carbohydrate

TABLE I

ANIMAL ASSAYS OF BIOLOGICAL ACTIVITY OF NATURAL AND SYNTHETIC CORTICOIDS

	A l e alectomi l log		A lrenalecto l ed rat						Adrenalectomi ed mouse
	Blood N P N	S du n retenti n	So l um retention	Uri e Na/K	Gro th surv al	Liver gl cogen	Anti inflammatory	Muscle work	Eosi ophils in blood
(a) Deso ycorticosterone	1	1	1	1 35	1	0·005	0	0 01	
Desoxycorticosterone acetate	?	1	1	1·00	1				
Corticosterone	0 5	0 18		0 14	0 2	0 36		0 3	
11 Dehydrocort co-sterone	0 1?5			0·07(ac)	0 15	0 ?8	1	0 24	
Hydrocortisone	0 04	?		0·08	0 ?5	1		1	1
Cort sone	0 04	?		0 06	0 5	0 5–1(ac)		0 5	1
Amorphous fraction of adrenal extracts	10							0 1	
Aldosterone	0	40	?5	1 0		0 33	0		0 3
(b)9α-Fluo ohydrocortisone	6 5	>2	0 7–5		<1	1?	7–13		10
Δ 9 Fluorohydro cortisone			–5			49	14–?5		
Δ Hydrocort sone			<1		1	3–5	3		3–4
Δ -Cortisone					1	3–5			3–4
Methylhydro cortisone		?	3			3–10	6	6	
2 Methyl 9 α-fluoro hydrocortisone		49	90			38	9	12	

I d at se of th ac tat I th case of th sy th ti d ri a s (b) th t t w re n a ly lw y sed Th d ta gi en th t bl re b tracted f m th f ll w g so rc B rn s Barn Bowma Dul n M ley a d S ff d (1956) D rfm n (1953) D l n (1955) Fri d (1955) G t Renz a d Ch rt (1955) H (195) H d (1950) L ddl Pech t d Bart (1954) L ddl R h d d T mkin (1956) N ble (1950) Sala d Ball b o (1956) S mpson a d T t (195–, 1955b) Staff d Barnes Bowma nd M nz g r (1955) Sw gl Bak E l L Bri d B k (1955) T lksd rf Bat Ca dy Macl od W rren nd P lm (19 6)

CHAPTER 9

NEW ADRENAL CORTICAL STEROIDS ALDOSTERONE AND SYNTHETIC COMPOUNDS

F T G Prunty

INTRODUCTION

In spite of its superficial nature the traditional classification of the biological effects of the adrenal cortical steroids still serves a useful purpose for as will be seen no compound has been developed which genuinely contravenes its generaliza tions The corticoids may be defined as steroids with a Δ^4 3 ketone structure and at least the substitution of a hydroxyl at carbon 21 and ketone at carbon 20 Their actions fall into two main groups (1) electrolyte and fluid balance sodium retention is the most widely known feature in this connexion and (2) certain functions in the regulation of carbohydrate metabolism There is indeed overlap of activity of individual compounds into each of these groups to a greater or lesser extent The adrenal cortex also synthesizes androgens which apart from having virilizing activity induce anabolism of nitrogen Since little advance has occurred recently in this particular field they will not be further considered in this chapter

The important active *natural corticoids* which are found in adrenal extracts or in the blood emerging from the gland are listed in Table I(a) The only recent addition to this list is aldosterone Another important advance is the development of a range of *synthetic substances* beginning with the synthesis of the 9 α halogen derivatives compounds which were found to have unexpectedly pronounced biological activity There is now in existence a range of active synthetic compounds (Table I(b)) which may be grouped into the following types (a) 9 α halogen deriva tives of cortisone and hydrocortisone for example 9 α fluorohydrocortisone (b) Δ^1 cortisone and Δ^1 hydrocortisone and (c) 2 methylcortisone and 2 methylhydro cortisone Interesting combinations of these substances have also been synthesized

Assessment of animal tests

Clinical assessment is not practicable unless something is known of biological activity and for this purpose a variety of tests are available which lend themselves to more or less accurate biological assay methods some of these methods have been the subject of reviews by Dorfman (1949 1953) and by Noble (1950) In Table I are set out eight methods of assay of corticoid activity in animals Slight differences in interpretation may appear owing to variations in technique used by different observers In considering the results of any bio assay procedure attention must be paid to the completeness and rapidity of absorption of the substance administered the duration of its action and the possibility of its destruction before the target organ is reached These considerations are particularly applicable to the corticoids ✓The tests tend to fall into two groups First ability to cause sodium retention in the adrenalectomized dog and rat There is difficulty in demonstrating sodium retention in these animals with substances such

serum potassium and rise in bicarbonate may be noted in data presented by Ward and Hench (1955) Qualitatively it appears to be as effective as desoxycorticosterone acetate in the maintenance of patients with Addison s disease but again the very small amounts of material available have prevented any long term observations of this effect The desirable dosage in man is about 100–200 micrograms daily Water retention occurs if the dosage is sufficient The effect may be observed in the few hours after administration of a dose or is manifest with sodium retention and weight increase when doses of 1 milligram daily are administered for 7 days in the case of Addison s disease (Thorn Renold *et al* 1955) Up to the pres nt no important effects have been observed in man on the blood pressure but that like desoxycorticosterone it is likely to cause elevation is suggested by the production of hypertension in rats when it is administered for several months (Kumar *et al* 1956) Since the effects in animals with respect to liver glycogen deposition are so weak it would not be expected that a 1 milligram dose in man would have any effect on indices of the carbohydrate regulating activity This proves to be the case with blood sugar levels anti insulin effect and nitrogen excretion

Blood eosinophil levels—In general there are no effects with the doses mentioned It is doubtful if the small depressions of eosinophils discussed by Thorn Renold *et al* (1955) can be regarded as significant and further observations on this point will be necessary

Corticotrophin measurement in the blood—A property which is usually linked with that of the carbohydrate regulating hormones is the ability to depress secretion of corticotrophin Since the measurement of corticotrophin in the blood is complex indirect methods of assessing the effect are employed One of the best methods is to observe depression of 17 ketosteroid excretion in patients with prepubertal virilism in whom the high 17 ketosteroid excretion is maintained by a high rate of corticotrophin secretion Cortisone is active in this respect in doses down to about 30 milligrams daily No effect was observed in one patient with doses of aldosterone of 1 050 micrograms daily (Prunty *et al* 1954) The same property may also be measured by observing the depression of the excretion of 17 hydroxycorticoids in normal individuals Thorn Renold *et al* (1955) found no suppression with daily doses of 1 milligram

Restoration of normal water handling—A function which has so far proved to be a property of carbohydrate regulating corticoids is the ability to restore normal water handling by the kidney after dehydration or water loading in patients with Addison s disease Aldosterone in the doses employed has not been demonstrated to correct this abnormality Thus aldosterone in an amount which may be regarded as physiological or in an amount up to 10–20 times this dosage behaves like desoxycorticosterone and has none of the properties of the carbohydrate regulating corticoids Only in excessively large doses in animals may these other effects be demonstrated In a brief trial Ward *et al* (1954) failed to demonstrate any antirheumatic properties in rheumatoid arthritis with daily doses of 1 000 micrograms or less ✓It has been estimated that in man aldosterone is from 20 to 50 times as potent as desoxycorticosterone

Physiology of aldosterone

The hormone has been demonstrated in the adrenal vein blood of the dog and rat and in minute amounts in the peripheral blood of man (*see* Dodds *et al* 1956) The

metabolism have an influence on the results Hydrocortisone is relatively much more active in this test than in the maintenance of the adrenalectomized dog The remaining group of tests show a different spectrum of steroidal activity and in each of the natural steroids hydrocortisone is the most active Inducement of deposition of liver glycogen in the adrenalectomized rat is a useful method of assay and is specifically related to the control of carbohydrate metabolism It will be seen that as in the case of the natural steroids synthetic steroids active in the test are also active in the muscle work test of Ingle (1944) the suppression of induced inflammation in rats and the depression of the eosinophils of mice In man liver glycogen levels cannot be easily determined and more indirect criteria have to be used such as eosinophil depression nitrogen catabolism increased urinary glucose and increase of uric acid excretion All these effects run parallel with the carbohydrate regulating activity of the steroids and for convenience these properties will be grouped together under that term It will also be seen that compounds which are active in this respect are potent in the depression of excretion of adrenal steroid metabolites when administered to patients

ALDOSTERONE NEW NATURAL CORTICOID

Indications that adrenal extracts contained potent biological activity which had not been accounted for by the known steroids they contained led to a search for new compounds (Simpson and Tait 1955b) With the aid of new methods of chromatography which have enabled use to be made of microgram quantities of material aldosterone was isolated and its chemical formula established Compared with other corticoid hormones it has a novel structure possessing an aldehyde group at C 18 (Simpson *et al* 1954)

Biological activity

Since the original isolation experiments were guided by a bio assay method which depended upon the ability of the substance to cause sodium retention and potassium excretion in the adrenalectomized rat its potency in this respect was already established It is shown in Table I that with regard to sodium retaining capacity and life maintenance in laboratory animals it is about 30 times as potent as the most active known steroid —desoxycorticosterone—and therefore active in the rat in amounts of 0 1 microgram or less On the other hand its effect on eosinophils and liver glycogen deposition is about one third that of cortisone There is no effect on suppression of inflammatory responses Indeed like desoxycorticosterone it may even antagonize the anti inflammatory effects of hydrocortisone when the granuloma pouch technique in the rat is used (Selye 1955) These findings have received almost complete confirmation in man Sodium retention can easily be detected with doses of 100 micrograms and this can be demonstrated in both normal individuals and patients with Addison s disease (Hetzel *et al* 1956) Its effect on potassium excretion is though transitory relatively intense so that the determination in the urine collected 3–5 hours after a dose of the sodium potassium ratio is a very sensitive measure of its activity Another convenient index of its activity is the decrease it produces in the sodium potassium ratio in the saliva (Simpson and Tait 1955a)

Dosage and effects

The substance is quite active orally but is best given intramuscularly (Hetzel *et al*, 1956 Thorn Sheppard *et al* 1955) The duration of its activity is only a few hours depending upon the dosage administered No pronounced changes in the electrolytes in the serum of patients given aldosterone have been observed probably because periods of observation have been short but the expected fall of

hyperplasia of the cortex Excess of aldosterone or sodium retaining substances in the urine may not be found but there may be evidence of excess urinary metabolites of both corticoids and ketosteroids An interesting feature is the way in which urinary ketogenic steroids may be increased together with elevation of the plasma hydrocortisone and without the development of typical Cushing s disease (Brooks *et al* 1957) On the other hand it has been realized for several years that hypokalaemia and alkalosis occur in Cushing s disease and a few patients with this condition have now been observed in which sodium retaining substances or aldosterone have been increased in the urine (Venning *et al* 1955 Nowaczynski *et al* 1956)

Primary aldosteronism

On account of the fact that Conn s patient did not have Cushing s disease and excreted excessive sodium retaining substance Conn labelled the condition primary aldosteronism This term was used to differentiate it from other pathological states in which excess aldosterone was known to occur The importance of this terminology lies in the fact that it stresses the peculiar clinical picture presented by these patients rather than indicating a constancy of hormonal pattern of the type originally suggested

Secondary aldosteronism

Before the chemical identification of aldosterone it was known that the urine of patients with subacute nephritis contained excessive amounts of a sodium retaining substance and this was later identified as aldosterone (Luetscher Dowdy and Harvey 1955) It has since been demonstrated in patients with cardiac failure with oedema and cirrhosis (Luetscher and Curtis 1955) These conditions have been termed secondary aldosteronism The mechanism is still enigmatical There is a reduction of urinary sodium excretion but hypokalaemia and alkalosis are absent due it is suggested to reduced sodium excretion through the kidney (Bartter 1956)

Hypertension and increased aldosterone excretion

Increased excretion of aldosterone may occur in other circumstances It has been observed in patients with hypertension (Gornall Gaulliam and Hall 1956 Genest *et al* 1956) briefly after surgical operations (Llaurado Neher and Wettstein 1956) and during stressful experiences (Venning and Dyrenfurth 1956)

Pregnancy

The excretion of aldosterone rises during the latter part of pregnancy as does the plasma hydrocortisone level (Martin and Mills 1956) Although at the moment there does not appear to be any additional increase of excretion in toxaemia of pregnancy the question of a possible role of aldosterone in this condition may not be entirely settled

Estimation of aldosterone

Since such small amounts of aldosterone are to be found in the urine it is probable that as in the case of hydrocortisone the major part of the hormone is metabolized to an inactive form Earlier investigations utilized methods of bio assay with inadequately

amount appearing in the urine in the form of free steroid after administration of a large dose intravenously in man is very small (Mills 1954) It seems likely that after allowances are made for conjugation the major part is metabolized to inactive steroids It is generally agreed that aldosterone secretion by the adrenal is only slightly stimulated by corticotrophin and in man the effect is small and immediate and tends to fall off after a few days administration (Bartter 1956) It is interesting to note that this elevation of aldosterone coincides with the period of maximal sodium retention when corticotrophin is given (Prunty *et al* 1953) Aldosterone may be found in the urine after hypophysectomy in dogs a reduction of about one third occurring (Farrell Rauschkolb and Koletsky 1955) it is present in the urine of patients with panhypo pituitarism and may be absent in Addison s disease

Secretion control

Its secretion can however be modified very greatly by changes in electrolyte ingestion and by hydration Reduction of sodium intake or increase of potassium intake stimulates its production and excretion (Luetscher and Curtis 1955) On the other hand alterations in the extracellular fluid volume are also effective reduction stimulating the secretion of the hormone This may be seen following the administration of Pitressin in man Aldosterone excretion falls with fluid expansion induced by Pitressin and rises after its withdrawal and when the volume of extracellular fluid is falling (Muller *et al* 1956) Bartter (1956) presented arguments that the effects of sodium are mediated through secondary changes in fluid volume and not by virtue of changes in electrolyte concentration whereas changes in potassium concentration may themselves exert an effect The latter is supported by the finding that perfusion of the adrenal with a potassium rich medium increases the amount of aldosterone produced by the gland (Rosenfeld *et al* 1956) That further mechanisms for the control of aldosterone secretion exist is suggested by the reduction in its concentration in the adrenal venous blood of decapitated dogs (Rauschkolb and Farrell 1956) There is experimental and other evidence to show that synthesis of aldosterone in the adrenal of the rat primarily occurs in the zona glomerulosa rather than in the zona fasciculata which is more com pletely under the control of corticotrophin (Giroud Stachenko and Venning 1956)

Pathological relationships of aldosterone

Hypokalaemia and alkalosis in Cushing s disease

Increases in the excretion of aldosterone have been noted under two differing types of circumstance In the first it appears as a result of pathological conditions of the adrenal gland These include benign and malignant tumours of the cortex and the over active adrenals of Cushing s disease This first came to light with the observation of Conn and Louis (1956) on a patient with an adenoma of the cortex It was shown that there was a large amount of sodium retaining substance presumed to be aldosterone in the urine although the excretion of 17 hydroxy corticoids and 17 ketosteroids was normal The chemical findings in this patient led to the discovery of similar cases Clinically there is hypertension in the absence of the typical obesity and muscular wasting of Cushing s disease together with a tendency to hypokalaemia and alkalosis The hypokalaemia may lead to incidents of muscular paralysis and even tetany as occurred in Conn s original patient Polyuria is also an important symptom Heart failure and even more important renal failure develop The latter may lead to confusion in the primary diagnosis and probably results from the hypokalaemia which is known to cause renal damage Patients observed since Conn s original description have shown deviations from this picture The pathological lesions may be adenoma carcinoma or merely

agree that their sodium retaining properties are intense and that the compound is active orally in small doses By slow intravenous administration 9 α fluorohydrocortisone is equipotent with aldosterone and its action may be prolonged (Thorn Sheppard *et al* 1955) This method of administration produces the most powerful effect on sodium retention whatever the steroid used in man hydro cortisone induces intense retention of sodium during the few hours of its infusion This may be due to the fact that rapid exposure of the kidney to a high hormone concentration in the blood permits increased tubular reabsorption of sodium before any effect in increasing its rate of glomerular filtration becomes predominant and obscures the increased reabsorption Judged in this way 9 α fluorohydrocortisone has almost 50 times the potency of the parent compound Like other steroids causing sodium retention these synthetic substances cause transitory increase in the excretion of potassium In a study in Addison s disease using more prolonged oral administration for several days Garrod *et al* (1955) obtained similar results After 7 or 8 days using 1 milligram doses there was a distinct tendency for decreasing sodium retention body weight increase due to fluid retention was pronounced and the expected fall in packed cell volume and serum potassium together with increase of serum sodium concentration occurred The salivary sodium potassium ratio fell

With regard to the properties associated with carbohydrate regulating activity *in man there is a similar unanimity in the results obtained Measurements of* urinary glucose excretion nitrogen loss and uric acid excretion led Thorn Renold *et al* (1955) to the conclusion that 9 α fluorohydrocortisone was at least 20 times as potent as hydrocortisone In Addison s disease Garrod *et al* (1955) noted a potency of less than 100 times that of cortisone Dosage was limited by the salt retention produced The abnormal T waves in the electrocardiogram in Addison s disease were not returned to normal with 0 5 milligram doses and the abnormal handling of water loads was not rectified to the same degree as with the cortisone in 50 milligram doses

Depression of adrenal activity

In individuals with normally functioning adrenals suppression of ketosteroids 17 hydroxycorticoids and 17 ketogenic steroids occurs with 9 α fluorohydro cortisone The reduction of the corticoid metabolites seems to be the greater of these two effects (Thorn Renold *et al* 1955 Cope 1956) In the adrenogenital syndrome 9 α fluorohydrocortisone is a powerful suppressor of the abnormally raised 17 ketosteroids and hence by inference of the rate of corticotrophin secretion (Kupperman *et al* 1955) This compound was the most powerful tested and was at least 10 times as active as cortisone so that doses of 2–3 milligrams daily were quite active The urinary 17 ketosteroids 17 hydroxycorticoids and 17 ketogenic steroids are only excreted in small amounts when 0 5–1 milligram of 9 α fluorohydrocortisone is given to patients with Addison s disease This advan tage together with its potency makes the compound eminently suitable for demon strating the depression of adrenal activity which can be induced in Cushing s disease due to adrenal hyperplasia The phenomenon is of practical use in the differentiation from adrenal cortical tumours which do not respond with such depression (Cope 1956) However it seems that occasionally when cortical hyperplasia is present (Prunty 1956) this lowering of ketogenic steroid excretion

purified urine extracts but recent developments have aimed at methods with more stringent criteria of physical and chemical purity (Neher and Wettstein 1956 Ayres Simpson and Tait 1957) Reports of the extreme range of this substance in normal urine vary from 0 to 19 micrograms per day (Avando *et al* 1956 Ayres Simpson and Tait 1957) Still further research is required for the development of reliable techniques

SYNTHETIC COMPOUNDS

The Halogenated Steroids

Electrolyte and carbohydrate regulating properties

The first of the new synthetic compounds to be widely studied were the 9 α bromo chloro and fluoro derivatives of cortisone and hydrocortisone The activity of 9 α fluorohydrocortisone in animal assays is indicated in Table I Enhancement of the electrolyte regulating properties has occurred so that it is approximately 5 times as active as desoxycorticosterone Surprisingly carbohydrate regulating properties with respect to hydrocortisone have also been enhanced about 10 times so that there appears to be a new balance of activity introduced into the compound by the presence of the fluorine atom The ratio of the electrolyte and carbo hydrate regulating potency is similar to that of corticosterone but the over all potency is about 30 times greater

Relative potencies the hydrocortisone series

Detailed studies have been carried out on the relative potencies of the halogenated series Fried (1955) compared the four halogenated hydrocortisones and bromo chloro and fluorocortisones In the hydrocortisone series a graduation was observed with respect to effects related to carbohydrate regulating activity in the direction iodo bromo chloro and fluoro 9 α fluorohydrocortisone being 100 times as active as the iodo derivatives and from 2 to 3 times as active as the chloro compound The hydro cortisone derivatives are seen to have about the same relative increase in potency compared to the cortisone derivatives as one might expect from the relative potencies of the parent compounds that is rather less than 2 to 1 This relative increase in potency was even more marked in the anti inflammatory test performed by the implanta tion of cotton pellets in the rat In the adrenalectomized dog 9 α fluorohydrocortisone acetate was more powerful in producing eosinopenia than the chloro compound (Liddle *et al* 1954) Comparison of the sodium retaining properties reveal interesting findings It was found that in low doses in the dog the effect on sodium retention exceeded that of desoxycorticosterone in the cases of the chloro and fluoro compounds and by approximately the same amount In higher dosage the increased sodium filtration induced by the increase of glomerular filtration rate resulted in over all loss of sodium In Fried s (1955) experiments on the other hand in the rat 9 α chlorohydrocortisone was the most potent steroid being about 10 times as active as desoxycorticosterone whilst 9 α fluorohydrocortisone was less active than this reference standard but others have found the fluoro compound to be 5 times as active as the reference substance in producing sodium retention in the rat (Stafford *et al* 1955) Differences in technique and species differences may account for these variations rather than any intrinsic dissociation of the properties of the steroids studied

Potency

In clinical observations similar conclusions have been drawn concerning the potency of the 9 α fluoro derivatives of cortisone and hydrocortisone All observers

Preliminary observations have been made in the rat (Fried 1955) and in man (Goldfien Morse *et al* 1955) From investigations of the biological effects of the known adrenal steroids the presence of a Δ^4 3 ketone grouping in ring A appears to be obligatory for activity to be evident However modification of the number and position of substituent hydroxyl groups may be conveniently observed by this method

The results of these studies are schematically summarized in Table II

It is interesting to note that the introduction of the 11 β hydroxyl group into progesterone produced some slight activity in increasing excretion of urinary glucose The potency of this compound becomes appreciable in all respects with the introduction of the 9 α fluorine atom although the activity was of a low order The addition of a 21 hydroxyl group further activates the compound in all respects whereas the 17 hydroxyl group tends to enhance carbohydrate regulating activity at the expense of mineral activity These conclusions are broadly in accord with the effects of variation of structures of the parent compounds (Table I) It is of interest to note the great sodium retaining effect of 9 α fluorocorticosterone The substitution of a methoxy or ethoxy group for fluorine in hydrocortisone abolishes activity in the rat

PREDNISONE AND PREDNISOLONE

These compounds are respectively Δ^1-cortisone and Δ^1 hydrocortisone which biologically appear to be almost indistinguishable from one another They are like hydrocortisone almost inactive in causing sodium retention in rats about equipotent with desoxycorticosterone in life maintenance and from 3 to 4 times more potent than hydrocortisone in carbohydrate and anti inflammatory effects (Table I) Modification of the hydrocortisone molecule therefore shifts the balance in favour of possible gain in anti inflammatory potency This again has proved to be the case and extensive work has already been done in patients The earliest metabolic studies (Thorn Renold *et al* 1955) showed with intravenous use a sodium retaining activity equal to that of cortisone but more prolonged When the substance was administered orally difficulty was encountered in the consistent production of sodium retention In a series of longer studies Nabarro *et al* (1955) found that sodium retention was difficult to induce and like others that a diuresis of water and sodium could indeed occur on changing from cortisone to prednisone On the other hand carbohydrate regulating effects were increased In an adrenalectomized patient the anti insulin effect was greater with prednisone than with cortisone and the eosinopenic effect in other patients was about 3 times that of cortisone The same authors comment too on the induced increase in nitrogen excretion This was also stressed by Thorn Renold *et al* (1955) who reached similar conclusions and by Bunim *et al* (1955)

Suppressive properties

These compounds are valuable in the suppression of adrenal cortical secretion suppressing corticoid metabolites and ketosteroids In adrenal virilism due to cortical hyperplasia the suppression is very good even in doses of 10 milligrams per day or possibly less (Kupperman *et al* 1955) Many surveys have been made in patients with rheumatoid arthritis (Hart *et al* 1955 Bunim *et al* 1955 Boland 1955 and others) and all agree on the potent effects There seems to be little difference between prednisone and prednisolone both being about 3–5 times as

can be *transitory* or even not occur Although doses of 1 milligram cause suppression of adrenal activity in normal individuals it is probable that doses up to 10 times this amount might be desirable in *diagnostic observations in Cushing's* disease (Cope 1956)

Therapeutic trials in rheumatoid arthritis

In therapeutic trials in rheumatoid arthritis and other conditions those who have used 9 α *fluorohydrocortisone* have found that salt and water retention are so pronounced that it necessitated abandonment of the study This may be accompanied by a significant fall of serum potassium and alkalosis (Ward and Hench 1955) An observation in Addison's disease on the comparative effects of 9α fluorocortisone and 9α chlorocortisone in inducing sodium retention indicates that the former is more active than the latter (Goldfien Laidlaw *et al* 1955)

It would be expected from the results of animal experiments and its metabolic effects indicating *pronounced carbohydrate regulating activity in human beings* that 9 α fluorohydrocortisone would have powerful effects in the control of rheumatoid arthritis and this is so Thirteen patients were treated by Boland (1955) and 4 by Ward and Hench (1955) The compound was found to be about 10 times as effective as hydrocortisone and cortisone but the observations had to be *terminated* on account of pronounced sodium and water retention Some significant effect in elevating the blood pressure was observed Other observers have reached the same conclusions

9 α Fluoro derivatives of related compounds

The ability to enhance the effect of steroids by the introduction of the 9 α fluorine atom has permitted a study of the effects of the alteration of structure in other parts of the molecule

TABLE II

EFFECT OF THE PRESENCE AND POSITION OF HYDROXYL GROUPS ON BIOLOGICAL ACTIVITY OF FLUORINATED STEROIDS

Compound	*Addition*	*Man*		*Rat*	
		Carbohydrate activity	*Mineral activity*	*Glycogen deposition*	*Sodium retention*
Progesterone	—	0	0	—	—
11 β Hydroxyprogesterone	11 β OH	±	0	—	—
9 α *Fluorine derivatives of* 11 β hydroxyprogesterone	11 β-OH	+	+	+	+
11 β 17 α Hydroxyprogesterone	11 β OH 17 α OH	++	+	++	±
Corticosterone	11 β OH 21 α OH	+++	++++	+++	++++
17 α Hydroxycorticosterone	11 β OH 17 α OH 21 α OH	++++	+++	++++	+

compounds was approximately the same as the parent compounds that is 2 methylhydrocortisone was as potent as hydrocortisone and 2 methylfluorohydrocortisone 10 times as potent Similar relations were found in respect of urinary nitrogen excretion Yet by the use of the granuloma pouch technique in rats 2 methyl 9 α fluorohydrocortisone in small doses could be shown to be more potent in the inhibition of the anti inflammatory action of hydrocortisone acetate than desoxycorticosterone acetate with larger doses this effect became less (Selye and Bois 1956) The metabolism of the methyl compounds is of interest for after their administration there is prolongation of elevation of blood levels when compared with hydrocortisone and their almost complete failure to contribute to the glucuronide fractions of 17 hydroxycorticoids About 3 per cent of 2 methylhydrocortisone can be accounted for in the urine as the free compound

Activity

A point of interest in these observations is the effect of 2 methylation on the activity of 11 β hydroxyprogesterone It will be recalled that 9 α fluorination activated the latter compound in carbohydrate regulating effects and sodium retention *Similarly 2 methylation of 11 β hydroxyprogesterone* renders it active in causing sodium retention

CONCLUSIONS

Corticoid activities

It has long been known that the Δ^4 3 ketone grouping in ring A is essential for biological activity of corticoids and that the 21 hydroxyl 20 ketone grouping is also necessary The activity of the 9 α fluoro and 2 methyl 11 β hydroxyprogesterones is therefore of interest The studies with the fluoro compounds confirm the impression that the addition of the 11 β hydroxyl group to the corticoid molecule enhances the carbohydrate regulating action with diminution of mineral activity and this is further accentuated by the addition of the 17 hydroxyl group Table III summarizes so far as can be judged from the published data the activities

TABLE III
APPROXIMATE RFLATIVE POTENCIES OF NEW STEROIDS IN MAN

Compound	*Electrolyte activity*		*Carbohydrate activity*	*Dose for adrenal suppression mg daily*	*Anti rheumatoid activity*
	Effective dose	*Potency DCA=1*	*Potency * F =1*		*Potency * E =1*
Aldosterone	100 μg i m	30	—?	?	0
9 α Fluorohydrocortisone	100 μg orally	20	10–20	2–5	10
Δ^1 Hydrocortisone	<100 mg orally	Very poor	3	10	4
Δ^1 9 α Fluorohydrocortisone	100 μg orally	20	?>3	1 5	?>10
2 Methylhydrocortisone	2 5 mg orally	1	1	4	—
2 Methyl 9 α fluorohydrocortisone	25 μg orally	100	10	6	—

F = hyd ocortison * E = cortisone

active as cortisone Oedema produced by the latter tends to disappear and sodium restriction is unnecessary Hypertension introduced by hydrocortisone may be reduced by these substances (Boland 1956) otherwise the side effects commonly seen with cortisone were apparent Boland particularly stressed the increased liability to peptic ulceration this seems to require further investigation

Prolonged action compared with cortisone

Some observations have suggested that the action of prednisone and prednisolone is prolonged in comparison with cortisone When similar doses in a comparison of cortisone and prednisolone were used the latter produced a higher blood level of steroid which persisted for a longer period (Ely Done and Kelley 1956) Studies of urine metabolites do not indicate that prednisone is metabolized to cortisone but rather that it is inactivated ultimately in the body by reduction of the 20 ketone grouping (Gray *et al* 1956)

Δ^1 9α Fluorohydrocortisone

This compound combines the features of a 9 α fluorohydrocortisone and prednisolone So far studies of its properties have been limited but it seems clear that it is the most active compound obtained as regards the deposition of liver glycogen and anti inflammatory tests a property which could be predicted from its structure (Table I) However further data are required before it is certain that its effect on liver glycogen is proportionately greater than its effect on inhibiting inflammation In both the rat (Table I) and man the sodium retaining properties are about equal to those of 9 α fluorohydrocortisone (Thorn Renold *et al* 1955 Prunty *et al* 1956) It is highly active in Addison s disease in doses of 250 micrograms orally daily It is however possible that the sodium retention may prove to be of shorter duration than in the case of 9 α fluorohydrocortisone (Li *et al* 1956 Sala and Ballabio 1956) In the normal individual in doses of 1–5 milligrams daily it has a good effect in the suppression of urinary ketogenic steroids and 17 ketosteroids (Prunty *et al* 1956) We have not enough data yet to evaluate its relative potency as an antirheumatic agent but it seems at least as potent as 9 α fluorohydrocortisone (Sala and Ballabio 1956)

2 Methylcorticosteroids

Potency

The latest introduction to the series of synthetic compounds are the methylated analogues (Liddle *et al* 1956) In the dog test (Table I) 2 methylcortisone or 2 methylhydrocortisone produced variable results on sodium retention whereas in the rat test the potency was slightly greater than that of desoxycorticosterone The surprising feature of these compounds is the great potency of 2 methyl 9 α fluorohydrocortisone (Table I) which exceeds even that of aldosterone On the other hand the sodium retaining activity of desoxycorticosterone is reduced by the process of 2 methylation In man 2 methylhydrocortisone appears to be more than twice as potent as hydrocortisone in producing sodium retention and potassium loss 2 methyl 9 α fluorohydrocortisone was active in 25 microgram doses In the glycogen deposition test (Table I) this compound appeared to be significantly more active than the parent 9 α fluorohydrocortisone and nearly as active as *Δ^1 9 α fluorohydrocortisone* In man the eosinopenic effect of the methylated

of androgenic product derived from cortisone use of smaller quantities of the fluoro compound may be desirable in replacement therapy after adrenalectomy for cancer or alternatively as an adrenal suppressive agent in patients upon whom it is not desired to operate In the latter case the need for avoiding excessive salt and water retention by sodium restriction and potassium chloride administration would have to be assessed Likewise 9 α fluorohydrocortisone is most useful for testing adrenal suppression but care should be exercised in hypertensive individuals with cardiac failure even if it is used only for a few days In such cases prednisone or prednisolone would probably be preferable In the adrenogenital syndrome for longer term suppression of adrenal activity the latter compounds are to be preferred (Kupperman *et al* 1955) although in the long run maximum therapeutic with minimal side effects might be obtained with combinations of small doses of Δ^1 fluorohydrocortisone and prednisolone In rheumatoid arthritis and other diseases suppressed by cortisone prednisone and prednisolone appear to be the most satisfactory steroids on account of absence of salt and water retention but for no other reason This property is useful with patients who have developed hyper tension and cardiac failure on cortisone as they can show considerable improve ment when prednisolone is substituted

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in man of the important compounds that have so far been discovered Increase in potency of the basic corticoids has been induced in two directions—increased mineral activity in the cases of aldosterone 9 α fluorination and 2 methylation and increased carbohydrate regulating properties by 1-2 dehydrogenation and 9 α fluorination

Aldosterone and related adrenal hormones

It has as yet proved easier to enhance electrolyte activity In the case of aldosterone in man it is doubtful if any carbohydrate regulating properties have been demonstrated owing to limitation of the doses used The discovery of aldosterone by no means precludes the possibility that other related adrenal corticoid hormones will be found further work remains to be done on the nature of its metabolism in the body and on the mode of regulation of its secretion At present its *field of practical usefulness appears to be restricted* as *other more* easily obtainable compounds produce the same effects Of the synthetic substances the extreme potency of 2 methyl 9 α fluorohydrocortisone in causing sodium retention is specially notable Its closest synthetic competitor appears to be 9 α fluorocorticosterone

Anti inflammatory activity

The practical demand now is not for powerful sodium retaining substances but for compounds with increased anti inflammatory activity and preferably *without* the capacity to induce salt and water retention and hypertension and without the other side effects usually associated with cortisone With the synthesis of pred nisone and prednisolone the former objective has largely been achieved but with little progress as regards the latter In the case of the fluorinated compounds the enhancement of all these properties simultaneously and in similar degree precludes their practical use for this purpose However the question is raised *of the* possibilities in new compounds of which there are bound to be many of dissociation of the properties grouped together as carbohydrate regulating So far there has been no major indication that this is likely to be so These properties run parallel with the anti inflammatory effects Possible clues such as the comment by Thorn Renold *et al* (1955) that aldosterone may exhibit a dissociation of the eosinopenic from other effects have been pointed out however and the powerful effects of the Δ^1 compounds in inducing negative nitrogen balance have been noted Liddle *et al* (1956) comment upon an apparent increase in potency of 2 methyl *hydrocortisone in the deposition of liver glycogen without a corresponding* alteration in its eosinopenic effect or in its urinary steroid depressing powers Δ^1 9 α fluorohydrocortisone and 2 methyl 9 α fluorohydrocortisone may show disproportionate potency in liver glycogen deposition

Most favourable therapeutic uses

At the present time the most favourable therapeutic uses for the available steroids may be summarized as follows In Addison s disease and after adrenalec tomy the continued use of cortisone is indicated for its maintenance value and induction of that ill defined sense of well being A few patients appear to require additional salt retaining steroids (Garrod *et al* 1955) and the oral use of 9 α fluorohydrocortisone in small doses is helpful In view of the small amount

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When equilibrium is established the space through which the substance or ion is distributed can be calculated At the present time these techniques have raised as many problems as they have answered and there is uncertainty as to the exact anatomical limits of for example the chloride space

Influence of other hormones and factors

The simplest method of studying the action of an adrenocortical hormone is to give it to an adrenalectomized or addisonian patient and observe the effect on electrolyte and water metabolism by the techniques described above It is now clear that the actions of a steroid under these conditions may be quite different from those in a normal person Cortisone will cause sodium and water retention in an addisonian patient but if the same patient is given desoxycorticosterone in addition cortisone may induce a diuresis of sodium and water Similarly Conn (1955) found that cortisone and hydrocortisone induced a natriuresis when given to a patient with an aldosterone secreting tumour The action of desoxycorticosterone may be qualitatively and quantitatively different in an addisonian patient and a normal subject In the former sodium and water retention with the development of pulmonary oedema is readily induced whereas in a normal subject—presumably because of the presence of other adrenocortical hormones—very large doses may fail to produce oedema and may in dogs give rise to polyuria and polydypsia with marked loss of potassium

These differences in action may be related not only to the antagonistic or modify ing effects of other hormones but also to the prevailing state of electrolyte and water balance Some investigators for example have found hydrocortisone to have little effect on sodium excretion whereas others observed that if the same experimental animal was given a similar dose and was also loaded with water the excretion of sodium actually increased Corticotrophin (ACTH) in a dose of 100 milligrams daily causes sodium retention in a normal subject on an unrestricted sodium intake when potassium acetate is given concurrently sodium retention does not occur This effect was attributed by Liddle Bennett and Forsham (1953) to displacement of body sodium by potassium ions and to sup pression of renal tubular exchange of hydrogen ions for sodium ions An alternative explanation may be that the potassium suppressed the secretion of aldosterone

SITE OF ADRENOCORTICAL HORMONE ACTIVITY

The obvious effect of adrenocortical hormones on renal function led some investigators to believe that the overall changes in water and electrolyte metabolism were attributable to this action the undoubted changes in intracellular composition were considered secondary to the alterations induced by altered renal function Studying the development of acute adrenal insufficiency in dogs Swingle noted several years ago that the external losses of sodium and water were too small to account for the observed degree of dehydration and haemoconcentration and postulated a shift of sodium potassium and water into the cells There is now increasing evidence that adrenocortical hormones have a direct extrarenal action and influence the exchange of electrolytes and water between the extracellular and intracellular compartments throughout the body Our knowledge of the wide spread influence of adrenocortical steroids in other physiological spheres makes this

CHAPTER 10

ROLE OF ADRENOCORTICAL AND PITUITARY HORMONES IN WATER AND ELECTROLYTE CONTROL

R I S BAYLISS

PROBLEMS OF INVESTIGATION

THE ROLE which pituitary and adrenocortical hormones play in controlling water and electrolyte metabolism is being elucidated only slowly This is due in part to the complexity of the problem and the many factors involved and in part to the fact that the pituitary and adrenal cortex secrete multiple hormones which may be antagonistic to each other There is also a lack of satisfactory methods for studying salt and water metabolism At one time investigators were content to study the concentration of ions in the plasma but very substantial losses or gains of sodium may occur without any measurable alteration in the plasma level Homeostatic mechanisms usually succeed in adjusting the volume of plasma water to maintain normal ionic values and osmotic pressure Thus in addisonian patients marked depletion of total body sodium can take place and even an addisonian crisis occur without the plasma sodium level falling Similarly in Cushing s disease the loss of potassium from the body may be considerable yet in the majority of patients the serum level is normal

Balance studies

An important step forward was the introduction of balance studies in which changes in body water are inferred from changes in weight and losses and gains of electrolytes determined by the difference between intake and output This technique assesses the external balance and only provides information concerning the overall exchanges between the body and the exterior as mediated in most instances by the kidney

There is no doubt that adrenocortical hormones influence the distribution of water and electrolytes between the extracellular and intracellular compartments The measurement of these internal shifts poses a considerable problem but is essential to a further understanding of adrenocortical action Direct chemical analysis of the cells is an imperfect solution of the problem In man only skeletal muscle is readily available for biopsy and it may not be representative of other tissues Furthermore the number of such biopsies must be limited There is also the practical difficulty of obtaining intracellular material free of extracellular fluid Despite these drawbacks analysis of skeletal muscle has been of value in confirming changes in intracellular ionic composition as determined by other methods

Further information about internal shifts of water and electrolytes has come from the use of dilution techniques in which a known amount of a foreign substance (such as inulin thiocyanate mannitol) or a radioactive isotope (sodium chlorine bromine potassium deuterium tritium oxide) is introduced into the body

When equilibrium is established the space through which the substance or ion is distributed can be calculated At the present time these techniques have raised as many problems as they have answered and there is uncertainty as to the exact anatomical limits of, for example the chloride space

Influence of other hormones and factors

The simplest method of studying the action of an adrenocortical hormone is to give it to an adrenalectomized or addisonian patient and observe the effect on electrolyte and water metabolism by the techniques described above It is now clear that the actions of a steroid under these conditions may be quite different from those in a normal person Cortisone will cause sodium and water retention in an addisonian patient but if the same patient is given desoxycorticosterone in addition cortisone may induce a diuresis of sodium and water Similarly Conn (1955) found that cortisone and hydrocortisone induced a natriuresis when given to a patient with an aldosterone secreting tumour The action of desoxycorticosterone may be qualitatively and quantitatively different in an addisonian patient and a normal subject In the former sodium and water retention with the development of pulmonary oedema is readily induced whereas in a normal subject—presumably because of the presence of other adrenocortical hormones—very large doses may fail to produce oedema and may in dogs give rise to polyuria and polydypsia with marked loss of potassium

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concept of renal and extrarenal actions more probable and *in vitro* studies suggest that steroids play an important role in regulating the permeability of cell membranes For example in a rather different field digoxin influences potassium exchange across the myocardial cell membrane In high concentration progesterone has a similar action but in low concentration it exerts its normal physiological effect on the endometrium where high concentrations of digoxin also have an action These differences may well be attributable to the stereochemical configuration of the particular steroidal molecule and to the different architecture of the cell membrane in the various tissues

The evidence for an extrarenal action of adrenocortical steroids is sound In adrenal insufficiency the composition of the faeces the sweat and the saliva changes in the same way as that of the urine Woodbury (1954) found in nephrectomized adrenalectomized rats that these hormones influenced the electrolyte composition of all tissues—an effect which must have been independent of renal action More recently studies of adrenal deficiency in dogs have shown a marked decrease in the extracellular volume (as measured by inulin mannitol or thiocyanate space) and a loss of sodium from this space that was greater than the net external loss in the urine and faeces This loss of sodium has been attributed to intracellular migration but analysis of soft tissues although showing an increase in water and potassium has not revealed any marked change in intracellular sodium concentration The studies of Hills *et al* (1953) have provided some important information on these events as they occur in adrenally deficient man Using the chloride and inulin spaces as a measure of extracellular fluid volume they found that the net loss of sodium potassium and water was greater than could be accounted for by renal excretion and that there were considerable exchanges between the extracellular and intracellular compartments In fact external water loss was not a

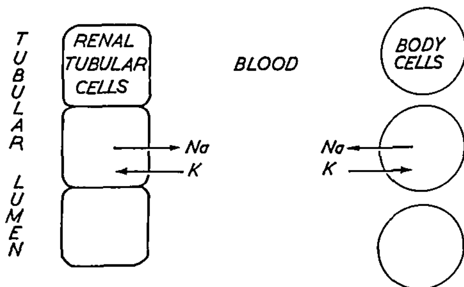


FIG 3 —Suggested action of an adrenocortical steroid on the transport of ions (*after Fourman*)

conspicuous or constant feature of acute adrenal insufficiency and internal transfers of sodium and potassium regularly preceded the development of hyponatraemia

These findings indicate that adrenocortical hormones produce a shift of water from inside the cells to the extracellular compartment At the same time there is a corresponding movement of sodium and chloride to the extracellular space while the transport of potassium into the cell is increased Fourman (1956) has suggested that the effect of an adrenocortical hormone on the kidney tubular cells is the same as on extrarenal cells because the steroid influences the cell surface in contact with the extracellular fluid and not the surface in contact with the luminal fluid (Fig 3) Thus the potassium content of the tubular cell is increased and the sodium content diminished and less potassium and more sodium are reabsorbed from the luminal fluid In the body the net gain of sodium and loss of potassium resulting from the action of the hormone on the kidney and the urinary excretion of electrolytes will ultimately overshadow the direct effect of the hormone on the body cells and will raise their sodium and lower their potassium content

HOMEOSTATIC MECHANISMS

The functional activity of a cell can only continue if the tissue fluid surrounding it is of the correct ionic composition and exerts within narrow limits the correct osmotic pressure Thus the composition of the extracellular fluid must ultimately be dictated by the physiological requirements and chemical constitution of the body cells themselves Little is known of these factors which in health remain comparatively constant since the ionic composition of the extracellular fluid as judged by analysis of the plasma also shows little variation This stability of the *milieu interieur* requires that the electrolyte concentration and the volume of the extracellular compartment be maintained within narrow limits and this is ultimately achieved by the kidneys

In laboratory animals adrenocortical hormones do not seem absolutely essential to these homeostatic mechanisms If kept at a constant temperature and fed on a high salt diet adrenalectomized animals survive and may breed On the other hand adrenalectomized patients cannot live without replacement therapy and the long survival of some addisonian patients given a high sodium low potassium diet before the advent of desoxycorticosterone (DOC) must be attributed to a small remnant of functioning adrenal tissue Some variation in tubular reabsorption of sodium is possible in adrenalectomized patients maintained on a constant amount of cortisone and DOC (Rosenbaum Papper and Ashley 1955) This indicates that fine adjustments of water and electrolyte control may not depend upon increased adrenocortical secretion and is in keeping with the general principle that these hormones do not initiate any reaction but impose an enhanced degree of flexibility and biological freedom by accelerating already existing processes

Control of concentration

Control of ionic concentration and hence of osmotic pressure in the extracellular fluid resides predominantly in the osmoreceptors which were first described by Verney and are discussed more fully in Chapter 8 A rise in the extracellular ionic concentration without a parallel increase in intracellular molar concentration causes the receptor cells to stimulate the posterior pituitary gland More antidiuretic hormone (ADH) is secreted and this in turn promotes increased reabsorp

tion of water by the renal tubules Conversely dilution of the plasma following a water load inhibits the release of ADH and the tubules reabsorb less water This response is dependent upon the functional integrity of the adrenal cortex In an addisonian patient given a water load the tubules continue to reabsorb almost the same amount of water as they did before dilution of the plasma occurred Although there is liability to water intoxication the excess fluid is eventually eliminated but at a rate less rapid than in the normal subject There have been several explanations for this failure of diuresis which is corrected by cortisone and the most probable is that in adrenocortical insufficiency an abnormally large proportion of the excess water passes into the intracellular compartment

The ability of the kidneys to reabsorb water in response to ADH secretion also depends upon the anatomical and functional integrity of the tubular cells Potassium depletion of the cells from any cause reduces their responsiveness to ADH and in primary hyperaldosteronism there is usually polyuria and hyposthenuria uninfluenced by injection of Pitressin This functional impairment of tubular activity is restored by replacement of the potassium stores which follows removal of the aldosterone secreting tumour

Control of volume

The distribution of fluid between the vascular interstitial and intracellular compartments of the body is governed by comparatively simple physicochemical processes which depend upon osmotic gradients Thus the relative volumes in the three fluid compartments are controlled mainly by ionic concentrations although—and this may be of some importance—changes in oxygen tension and *p*H also cause movement of water into and out of the cells There must however be some mechanism other than the osmoreceptors and ADH for controlling the total amount of fluid in the body because an infusion of isotonic saline solution into a normally hydrated subject is followed by increased renal excretion of salt and water until the normal volume has been restored Here again the kidney is the effector organ and maintains the fluid volume by adjusting the excretion not only of water but also of electrolytes There is evidence for believing that the kidney does this primarily by regulating electrolytes (particularly sodium) rather than water Thus enhanced tubular reabsorption of sodium is accompanied by increased water retention whereas to reduce the volume of an excessive amount of isotonic fluid the excretion of sodium is increased and this is associated with loss of water

It has been supposed that analogous to the osmoreceptors are the volume receptors which moderate renal function The site and nature of these hypothetical cells is unknown Bull (1953) suggested that the stimulus to the volume receptors is an alteration in the circulation because postural changes from the recumbent to the upright position the application of cuffs to the thighs haemorrhage and congestive cardiac failure are all associated with diminished excretion of sodium and water He postulated that the volume receptors are either sensitive to pressure changes in one of the fluid compartments or alternatively are chemoreceptors activated by circulatory inadequacy There is evidence that some volume receptors sensitive to changes in intrathoracic pressure are located in the right side of the heart and that the afferent impulses are transmitted by the vagi The efferent impulses from the medulla are in part at least humoral because renal transplantation or denervation does not interfere with the ability to regulate the

fluid volume The nature of the humoral agent or agents is uncertain but there is increasing evidence that by its action on sodium and potassium metabolism aldosterone may play an important part in the maintenance of fluid volume

ALDOSTERONE

Of the many steroids elaborated in the adrenal cortex hydrocortisone corticosterone and aldosterone are the most important in man according to present knowledge Although there is considerable qualitative overlap in their biological activity it is convenient to classify aldosterone as a mineralocorticoid (exerting its effect on sodium potassium and water metabolism) and hydrocortisone as a glucocorticoid with a wide spectrum of activity in many different spheres but having some effect on electrolyte metabolism and playing an important role in the control of ionic concentration Corticosterone has been less extensively studied but appears to occupy in its action an intermediate position between hydrocortisone and aldosterone Although some adrenalectomized patients can be maintained in health with hydrocortisone or cortisone alone biochemical normality is not achieved Similarly adrenalectomized patients can be maintained on aldosterone alone but judging by their inability to diurese a water load they too are not normal and hydrocortisone is an essential addition for the avoidance of water intoxication It is therefore impossible to ascribe maintenance of water and electrolyte homeostasis to one or other group of adrenocortical hormones However aldosterone is many times more potent than hydrocortisone in maintaining sodium balance in an adrenalectomized dog (10 micrograms a day being equivalent to 5 000 micrograms of hydrocortisone) and it plays the more important part in controlling the fluid volume of the body

Low dosage effects

The amount of aldosterone required to restore sodium and potassium balance in an addisonian or an adrenalectomized patient is about 100-200 micrograms per 24 hours It would be unwise however to assume that this quantity is secreted daily by a normal person since the action of the hormone may be modified by the simultaneous secretion of hydrocortisone Aldosterone in low dosage has a powerful effect in promoting the renal conservation of sodium and a less impressive action on the elimination of potassium in adrenalectomized animals Water retention and improvement in hydration occur secondary to the sodium retention As would be anticipated internal shifts occur Prunty *et al* (1954) found in an addisonian patient that despite the improvement in sodium balance the serum sodium concentration continued to fall and the body weight and the chloride space increased slightly These findings suggest that there was migration of water from the intracellular to the extracellular compartment

High dosage effects

The action of aldosterone in high dosage over a prolonged period of time can be assessed from the abnormalities that develop in patients with an aldosterone secreting adrenal tumour The biochemical findings are hypokalaemia (which is often responsible for the presenting symptoms) a minor or insignificant degree of hypernatraemia hypochloraemia and alkalosis The renal disturbances of inability to excrete an acid urine isosthenuria and polyuria are secondary to the disordered

function of the tubular cells resulting from potassium depletion The outstanding disturbance caused by the excess of aldosterone *per se* is potassium loss and this may be secondary to sodium retention and replacement of intracellular potassium by sodium ions In such patients a high potassium intake induces a negative sodium balance—the intracellular sodium being displaced by the potassium

Pituitary control of aldosterone

For the last three decades endocrinological thought has been dominated by the concept that the pituitary gland is the conductor of the endocrine orchestra and it was natural to suppose that the secretion of aldosterone was primarily controlled by some trophic hormone elaborated in the anterior hypophysis However in respect of electrolyte regulation the independence of the adrenal cortex from pituitary control has long been suspected on clinical grounds Serious sodium depletion or an addisonian crisis due to a reduced plasma volume seldom if ever occurs in panhypopituitarism In hypophysectomized animals there is atrophy of the inner two zones of the adrenal cortex but the glomerulosa layer remains intact These two observations suggest that aldosterone is formed in the outer layer of the cortex and that its formation is largely independent of pituitary control Further evidence for this comes from the finding that whereas negligible amounts of aldosterone are excreted in Addison s disease normal amounts may be found in the urine of a patient with panhypopituitarism Furthermore under the stress of sodium depletion a recently hypophysectomized patient excretes increased amounts of aldosterone but this may not occur in a long standing case If this finding is confirmed it suggests that some pituitary trophic factor is required to maintain the responsiveness of the aldosterone secreting cells but it need not imply that such a factor regulates secretion Findings which suggest independence of control by corticotrophin are the consistent diurnal variation in the excretion of 17 hydroxy corticosteroids without parallel variation in aldosterone output and the failure of aldosterone to cause pituitary suppression as judged by a diminished excretion of 17 ketosteroids or glucocorticoids Nor is aldosterone output suppressed by administration of hydrocortisone The effect of injecting corticotrophin on aldo sterone secretion is also uncertain The response if any is small compared with the increased output of 17 hydroxycorticosteroids and 17 ketosteroids even allow ing for the normally low aldosterone to hydrocortisone ratio The consensus of opinion is that small increases may occur but these are insignificant compared with the changes induced by manipulation of electrolytes and water

Water and electrolyte control of aldosterone

There is much evidence that the secretion of aldosterone is in some way regulated by the electrolyte or water content of the body but changes in sodium potassium and water are so closely related that it is difficult to determine experimentally which is the primary controller In man a reduction in dietary sodium or loss of sodium from the body causes increased aldosterone secretion but such manipula tions of sodium balance will also be accompanied by changes in the body fluid volume In dogs sodium depletion causes increased aldosterone secretion only when associated with an increased intake of potassium Some investigators have found that the restriction of sodium in man even when accompanied by a fall in serum sodium concentration is followed by little change in aldosterone production

unless potassium is provided in the diet In rats the weight of the glomerulosa layer of the adrenal cortex correlates better with changes in the serum potassium level than in the sodium level There are other indications that potassium may influence aldosterone production quite independently of changes in sodium or water balance Bartter (1956) has found that subjects on a sodium free diet who cannot therefore accumulate more sodium show a reduction in aldosterone secretion when also deprived of potassium Conversely when potassium is given to such a subject and sodium loss is prevented by simultaneous readministration of the same amount of sodium as is excreted in the urine aldosterone output rises It is interesting to note that the yield of aldosterone from a perfused beef adrenal gland is enhanced by a perfusate of high potassium content (Rosenfeld *et al* 1956)

The regulation of aldosterone secretion has also been attributed to changes in fluid volume Acute sodium depletion accompanied by water loss is associated with increased aldosterone production but if any change in fluid volume is prevented by water replacement there is little alteration in aldosterone output Increases in fluid volume induced by injections of Pitressin are associated with a fall in aldo sterone secretion despite a reduced serum sodium concentration and increased urinary excretion of sodium (Liddle *et al* 1955 Beck *et al* 1955 Muller *et al* 1956) Conversely a reduction in fluid volume unassociated with sodium depletion causes a rise in aldosterone output Bartter *et al* (1956) have evidence that it is changes in the extracellular fluid volume and not the intracellular volume which modify aldosterone secretion Thus a reduction in total body fluid is a less potent stimulus than a reduction in extracellular volume and intravenous administration of hypertonic saline solution to a sodium depleted subject causing expansion of the extracellular compartment but contraction of the intracellular volume reduces aldosterone formation

At present it must remain undecided whether a change in potassium content or fluid volume is the prime mover in regulating aldosterone secretion Both explana tions still present many difficulties If fluid volume changes prove to be of major importance the mechanism by which the adrenal cortex is stimulated or depressed is still unknown One of the difficulties in accepting changes in fluid volume as of primary importance is that despite considerable increases in extracellular fluid volume increased aldosterone secretion is found in oedematous states whether caused by cardiac renal or liver disease Yet patients with primary hyper aldosteronism due to an aldosterone secreting tumour do not develop oedema despite marked sodium retention no doubt this is due to the excess sodium being stored in the cells where it replaces potassium ions Patients with adrenal insufficiency on the other hand do develop oedema if given 800 micrograms of aldosterone daily over a period (Salassa *et al* 1954) and it seems therefore that in Addison s disease absence of other adrenocortical hormones prevents the retained sodium being stored in the cells it remains in the extracellular compart ment with accompanying water retention

In oedema water retention is secondary to sodium retention and the sodium retention is due to increased sodium reabsorption by the renal tubules This increased reabsorption cannot be ascribed solely to a reduction in the glomerular filtration rate and appears to be an isolated alteration in tubular activity since other tubular functions remain normal Many studies now confirm that in oedematous conditions there is an increased excretion of a sodium retaining

substance recently identified as aldosterone which is presumably responsible for the enhanced reabsorption of sodium Although much attention has been focused on renal tubular function and the action of aldosterone it must be remembered that the primary disorder causing the oedema resides in the liver kidney or heart and that some intermediate mechanism must operate to cause increased aldosterone secretion

THE ADRENAL GLANDS AND OEDEMA

There is evidence that the adrenal glands do play a major and perhaps essential role in the genesis of oedema Adrenalectomy prevents the recurrence of oedema in patients with congestive failure due to hypertensive heart disease and prevents or abolishes it in experimental cardiac failure produced by constriction of the pulmonary artery (Davis *et al* 1955) It is also necessary to seek the reason why in secondary hyperaldosteronism associated with oedema there is sodium retention without significantly increased excretion of potassium The explanation probably lies in the tubular mechanism for the excretion of potassium Potassium is excreted in exchange for other cations particularly sodium In secondary hyper aldosteronism sodium is so avidly reabsorbed from the glomerular filtrate that in the distal part of the renal tubule little is available for exchange with potassium Furthermore most of the retained sodium remains in the expanded extracellular space and does not replace intracellular potassium

It is also clear that even before oedema develops profound and ill understood changes in intracellular osmolarity and metabolism occur as a result of circulatory insufficiency in cardiac disease and other conditions particularly disorders of the liver and the kidneys The striking biochemical abnormality is the development of hyponatraemia for which treatment with infusions of sodium only leads to the development of oedema The cause of this type of hyponatraemia is uncertain but clinically it is of evil omen although improvement in the underlying disease process is accompanied by a gradual restoration of normal serum sodium levels The speed of onset of hyponatraemia is such that its occurrence can only be attributable to some profound alteration in cellular metabolism For example a previously healthy man known to have a normal serum sodium level of 145 milliequivalents per litre suffered an extensive myocardial infarct at 10 a m He received no treatment whatsoever and on admission to hospital 6 hours later during which time he had formed no urine his serum sodium level had fallen to 118 milliequivalents and there was no hyperkalaemia This hyponatraemia cannot be due to sodium depletion and can only be attributed to shifts in water and sodium between the intracellular and extracellular compartments Many workers have reported similar findings under a wide variety of conditions particularly in patients who have undergone cardiac surgery The hyponatraemia has been attributed to a primary lowering of the intracellular osmotic pressure the extra cellular tonicity being maintained at a subnormal level to conform with that of the intracellular fluid Over a period of days there is a substantial loss of potassium from the cells and in some instances the administration of potassium has raised the serum sodium level Administration of saline solution causes only a temporary increase in the serum sodium level which is coupled with intense thirst and is followed by a return of hyponatraemia with the development of oedema Although speculative it may prove that these changes in intracellular metabolism are due to

hypoxia Consideration of the speed of onset and the fact that similar cellular changes occur in adrenalectomized animals (MacPhee 1955) make it unlikely that alteration in adrenocortical function is the cause of the hyponatraemia

A SODIUM LOSING HORMONE

No discussion of the role of adrenocortical hormones in control of salt and water metabolism would be complete without reference to the possibility of a sodium losing hormone Some support for the existence of such a substance which would compete with a sodium retaining hormone is given by the isolation of a factor from the urine during a corticotrophin induced diuresis which promotes in animals *the elimination of sodium* There is also evidence that such a substance may be formed in some patients with congenital adrenal hyperplasia In this disease there is a defect in the biosynthesis of 17 hydroxycorticosteroids This causes increased secretion of corticotrophin and the adrenal glands respond by producing large amounts of either normal androgens or abnormal virilizing compounds About 10 per cent of such patients have a sodium losing syndrome which results in a state similar to that found in Addison s disease The inability to conserve sodium might be due to failure to synthesize aldosterone or to the formation of a salt losing compound In support of the former hypothesis is the report that aldosterone is absent from the urine of such patients (Luetscher 1956) In support of a salt losing hormone is the observation by Jailer that infusion of corticotrophin aggra vates the condition and the finding of aldosterone in the urine before corticotrophin is given (Prader Spahr and Neher 1955)

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CHAPTER 11

ADRENAL CORTICAL AND OTHER FACTORS IN HYPOPITUITARY COMA

J E CAUGHEY

INTRODUCTION

THE ASSOCIATION of hypopituitarism with disturbances of consciousness has been recognized for many years and there must be few clinicians with experience of dealing with this condition who have not observed the attacks of drowsiness stupor and loss of consciousness which periodically occur in such patients As an end result death is likely to supervene in coma

Cushing (1912) stated that it had been shown experimentally that animals deprived of a considerable portion of the pituitary gland suffered a marked fall in body temperature and that this state was apt to be accompanied by profound somnolence He correlated the syndrome of hypopituitarism in animals with the clinical syndrome as observed in man and emphasized the anterior lobe deficiency He noted the skin changes the hypothermia the apathy the disturbances in carbohydrate metabolism and the gonadal atrophy associated with this condition Some of the patients on whom he reported had attacks of unconsciousness while others had disturbances of consciousness such as hypersomnia and torpidity He found that a tendency to somnolence was characteristic of almost all cases in his series and that many were inclined to doze throughout the whole 24 hours In other patients somnolent periods occurred in cycles with intervening days of fairly normal responsiveness

In one of Simmonds (1914) original cases hypopituitarism followed puerperal sepsis The full picture developed over the years and prior to admission to hospital the patient became increasingly drowsy finally passing into coma The patient did not regain consciousness and died the day after admission Similarly Simmonds (1918) second case was a woman aged 48 years who was admitted to hospital in coma and died without regaining consciousness Studying the records in the literature of hypopituitarism Sheehan and Summers (1949) discovered 96 cases of whom 62 died in coma The coma was usually attributed to hypoglycaemia Further study however has shown that hypoglycaemia is only one of many factors which may affect the state of consciousness in hypopituitarism Allott and Simmons (1951) described a patient with hypopituitarism due to post partum necrosis of the pituitary who was in deep coma There was little response to desoxycorticosterone acetate and infusion of glucose in saline solution although after 48 hours the blood sugar had returned to normal Within 24 hours of starting cortisone therapy a marked improvement set in Sheehan and Summers (1952) studied 9 patients with hypopituitarism due to post partum necrosis 8 of

whom passed into coma They found that the coma was sometimes associated with hypoglycaemia sometimes with low plasma sodium and chloride and sometimes with severe hypothermia which they stated might easily pass unrecognized

Caughey James and Macleod (1952) described 4 patients with hypopituitarism and pituitary tumour who were operated upon following hypophysectomy they had varying disturbances of consciousness *Ingraham Matson and McLaurin* (1952) reported hypopituitarism due to craniopharyngioma associated with coma fever and vomiting Gordy Peet and Kahn (1949) reported a post operative mortality of 41 per cent in 51 cases of craniopharyngioma and they attributed the high mortality to hypothalamic damage as a result of operation Northfield (1955) considered that severe reactions following operation for pituitary tumour and craniopharyngioma were usually due to damage to the vegetative centres

Caughey and Garrod (1954) reported on 17 patients with hypopituitarism all of whom showed disturbances of consciousness which varied in severity from mild confusion hypersomnia defective cerebration and stupor to profound coma The precipitating factors were apparently variable and included operations in the vicinity of the pituitary fossa pituitary apoplexy infections hypoglycaemia drugs anaesthetics *alcohol electrolyte disturbance water intoxication and cerebral* anoxia

It is evident from such reports that patients with hypopituitarism are unduly vulnerable to a number of factors which affect the level of consciousness and that coma may supervene and be a serious medical emergency

FACTORS PRECIPITATING CHANGES IN CONSCIOUSNESS

Surgery of the pituitary gland and neighbouring areas

Out of 71 patients with intrasellar tumours reported by Grant (1948) 91 per cent had evidence of hypopituitarism In a series of 85 operations in these cases 8 deaths occurred (11 2 per cent) Six of these cases were described as burned out chromophobe adenomata with polyglandular function at a very low ebb It was recorded that following relatively simple operations in 3 of the cases the patient became stuporous on the fifth day comatose on the ninth day and died on the twelfth day after operation Grant commented a simple evacuation of the tumour was enough to abolish completely what little pituitary function remained His belief was that post operative mortality in cases of *hypopituitarism was high* and that surgical attack should only be undertaken with much caution

Gordy Peet and Khan (1949) in 51 cases of craniopharyngioma had an operative mortality rate of 41 per cent due principally to severe hypothalamic reactions Grant s (1948) operative mortality rate in 40 operations on 30 patients of this type was 35 *per cent and he noted generalized endocrine atrophy in many of* the fatal cases He commented on the dangers of hyperthermia from hypothalamic damage and complete glandular collapse after operation In an attempt to reduce the high post operative mortality rate Roche Thorn and Hills (1950) investigated adrenocortical function on the grounds that this mechanism might be an *important factor They utilized the eosinophil levels during and after surgery* and the response to corticotrophin as a method of assessing this function They considered that a fall in the eosinophils after an injection of 25 milligrams of corticotrophin prior to surgery was a good index of the capacity of the adrenal

cortex to secrete 11 oxysteroids and that by this test a good measure of cortical function could be obtained

Caughey James and Macleod (1952) reported 3 patients who passed into pro longed coma following operation for pituitary tumour and Caughey and Garrod (1954) reported another case In 2 of these there was clearly evidence of thyroid and adrenal failure After operation neither patient regained consciousness but remained in a stuporous and comatose condition for several days In 1 case this lasted for 6 weeks before recovery These authors considered that the responsible factor was adrenal failure Northfield (1955) on the other hand was of the opinion that severe reactions following operation for pituitary and Rathke pouch tumours were usually due to damage to the brain stem He considered that adrenal failure was only a rare cause

Pituitary haemorrhage

The condition of pituitary haemorrhage or pituitary apoplexy as it is so called is one which will seldom be recognized clinically though it is not unusual to find haemorrhagic fluid in cysts in pituitary adenomas at necropsy examination Aitken and Russell (1934) called attention to the symptoms and signs which might be expected as a result of such haemorrhage and they also reported the case of a man aged 50 years with well marked hypopituitarism who died of this condition A few hours after the onset of dizziness weakness and vomiting the patient became semi conscious and remained so for 3 days On recovery he was apathetic and drowsy a state which continued for some 41 weeks until he died At necropsy a chromophobe tumour of the pituitary with an intrasellar haemorrhage was found The author has had experience of 2 other such cases one of which has been recorded in detail (Caughey and Garrod 1954)

Intercurrent infection

As has been said hypersomnia is a feature of hypopituitarism and in the presence of an intercurrent infection it may be more pronounced In fact such a condition may be the trigger factor of an acute medical emergency Caughey and Garrod (1954) reported that infections such as colds influenza and gastro enteritis had caused drowsiness confusion and eventually coma in 8 of their patients In 1 and within 3 months of a severe post partum haemorrhage coma developed during acute pyelonephritis pituitary necrosis was confirmed at necropsy

Hypoglycaemia

In hypopituitarism hypoglycaemia may occur spontaneously during infections or following injections of insulin given either for therapeutic purposes or in the performance of the insulin tolerance test There may also be a tendency in this condition to reactionary hypoglycaemia after high carbohydrate meals Oliver (1952) reported a patient not recognized as having hypopituitarism but who in fact had a tuberculoma of the pituitary gland he died in coma after 2 injections of 10 units of insulin which had been given to promote appetite Fraser and Smith (1941) stressed the value of the intravenous insulin tolerance test in the diagnosis of hypopituitarism but they gave warning about its possible dangers and advised using only one third of the standard dose of 0 1 unit of insulin per kilogram of body weight when severe hypopituitarism was suspected Rolland and Matthews

(1952) reported severe hypoglycaemic symptoms in a man with hypopituitarism after 1 5 units of insulin and Hausmann *et al* (1951) induced hypoglycaemic shock with 1 75 units of insulin in a patient with hypopituitarism due to post partum necrosis This patient remained in a state of semi coma for 2 weeks despite energetic treatment There is much evidence therefore to support the view that in hypopituitarism the insulin mechanism is hypersensitive

Hypersensitivity to drugs and anaesthesia

In hypopituitarism hypersensitivity to drugs is comparable to that found in Addison s disease In a case of the author s an injection of ¼ grain (16 milligrams) of morphine sulphate prior to an air encephalogram induced coma which lasted for 2 days In another case coma followed quinalbarbitone 1½ grains and pethidine 50 milligrams A further patient passed into the third stage of anaesthesia after pentobarbitone sodium 1½ grains (0 1 gramme) and less than 4 millilitres of intravenous thiopentone sodium this patient remained in a state of semi coma for the following 2 days Decreased tolerance to alcohol is another feature of hypopituitarism A good illustration is a patient who prior to hypo physectomy for carcinoma of the breast could take 3 or 4 gins without being affected whilst after operation 1 small gin made her so drowsy that she was unable to lunch with her guests and had to go to bed where she slept for 3 or 4 hours

Sodium depletion

It is well known that sodium depletion is a constant finding in untreated Addison s disease and it could be anticipated that a similar sodium depletion might occur in certain cases of hypopituitarism owing to the possibility of corticotrophin deficiency and secondary adrenal failure The isolation of aldosterone (Simpson *et al* 1954) with its predominant electrolyte effect has helped to clarify the relation ship of the adrenal cortex to sodium and potassium metabolism It is to be noted however that some reports (Cope and Llaurado 1954 Luetscher and Axelrad 1954 Axelrad *et al* 1954) suggest that aldosterone secretion may be largely independent of corticotrophic control and this if confirmed may explain why some hypopituitary patients show little indication of sodium depletion Neverthe less it does occur in hypopituitarism and with resulting coma and it may be provoked by gastro intestinal upsets or even by cortisone therapy One patient reported by Wynn and Garrod (1955) passed into deep coma with a markedly negative sodium balance within a week of starting cortisone therapy In Addison s disease and in hypopituitarism sodium excretion may rise greatly during the first day or two of cortisone therapy The same phenomenon occurs in the adrenalecto mized dog and it appears to be due to a rise in the glomerular filtration rate (Garrod personal communication) Cases with a sodium losing crisis leading to coma have been reported in hypopituitarism by Laws (1952) and by Caughey and Garrod (1954)

Water intoxication

Patients with hypopituitarism and secondary adrenal failure may be unable to excrete water at the normal rate and retention of water may lead to their death

cortex to secrete 11 oxysteroids and that by this test a good measure of cortical function could be obtained

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showed that treatment with cortisone acetate and *l* thyroxine in combination restored normal cold adaptation to hypophysectomized rats He believes that chronic sensitivity to cold in these animals is due to adrenal cortical and thyroid inactivity with resultant deficiency of corticosterone and thyroxine

In the past improvement in the general condition of patients with hypopituitarism has been noted after treatment with desoxycorticosterone and thyroid With the addition of cortisone to the physicians armamentarium the results of full substitution therapy in uncomplicated cases have been dramatic Patients who have been almost completely incapacitated by mental dullness and torpor asthenia and cold sensitivity have been soon restored to normal so confirming the experimental findings of Hornabrook It would appear therefore that the disturbance of consciousness in hypopituitarism in human beings can be ascribed to lack of corticotrophin and thyrotrophin with resultant deficiency of cortical hormones and thyroxine These lead to impaired function of the critical areas of the brain which are concerned with the maintenance of consciousness

DIAGNOSIS

In any case of obscure coma the possibility of hypopituitarism should be considered Certain general features of the patient may suggest this underlying condition It may too be possible to get a previous history from relatives and previous symptoms of hypersomnia asthenia sensitivity to cold loss of libido and coma would be clearly suggestive in a woman the diagnosis is supported by an earlier history of post partum haemorrhage or puerperal sepsis with subsequent amenorrhoea or scanty menses culminating in a premature climacteric If the hypopituitarism was due to tumour there might be the further history of neigh bourhood symptoms and signs headache visual failure and primary optic atrophy Such features would call for radiography of the pituitary fossa The diagnosis of hypopituitarism will not be further considered here as full details can be obtained in text books of medicine It might be mentioned however that on examination of a comatose patient certain features such as pallor a soft thin skin myxoedema tous changes loss of body hair with genital atrophy would point to hypo pituitarism

In hypopituitary coma the condition may be ushered in by a period of drowsiness prolonged sleep confusion disorientation or slurred speech In the state of stupor the patient can usually be roused by shouting or by painful stimulation although there is often a relative insensitivity to pain The superficial and deep reflexes are often retained the temperature may be normal but according to Sheehan and Summers (1949) it is usually low and between 95 F and 97 F Investigations must be carried out to clarify the biochemical state Hypoglycaemia may be present and it must be remembered that a state of coma from this cause may persist long after the blood sugar is restored to normal On recovery there is often amnesia for the duration of the coma One further note of warning is perhaps necessary about the risks of an insulin tolerance test in hypopituitarism The inherent dangers have already been discussed and hypoglycaemic shock may pass on to hypopituitary coma—which may even be fatal this test should only be used with the greatest caution and should not be employed if the necessary information can be obtained by other means A combination of hypotension azotaemia and

in coma (Whittaker and Whitehead 1954) This complication may arise spontaneously or even as a result of a water excretion test when the plasma sodium concentration is already low It is a potential danger in the diabetes insipidus of posterior lobe deficiency if anterior pituitary function becomes impaired as well and in certain compression lesions or after hypophysectomy

Cerebral anoxia

Hypotensive postural disturbances are also likely to occur in hypopituitarism they may result in faints from cerebral anaemia or even in a convulsive attack A state of drowsiness and stupor was noted by one of the author s patients both during and after travel in a commercial aircraft flying at a height of only 5 000 feet

Hypothermia

In hypopituitarism the individual is intolerant to cold as occurs in myxoedema and he may become drowsy and mentally confused during cold weather In one reported post operative case the temperature fell to 96 F (35 6 C) and on warming for 6 hours the temperature rose to 99 F (37 2 C) but there was no return of consciousness (Caughey and Garrod 1954) In a case of pituitary tumour with hypopituitarism (Decourt *et al* 1954) hypophysectomy was carried out using hypothermia for an anaesthetic The patient passed into the third stage of anaesthesia and failed to recover

THE MECHANISM OF COMA IN HYPOPITUITARISM

Our understanding of the mechanism of coma has been advanced by recent studies of neuronal metabolism Consciousness which has been defined as an awareness of environment and of self (Cobb 1948) has been shown to be dependent upon the integrity of certain critical areas within the cerebral cortex the thalamus the hypothalamus and the reticular area of the mid brain (Cairns 1952) Any disturbance of neuronal metabolism within these centres of consciousness may affect the mental state The metabolism of neurones is concerned chiefly with oxidation of glucose and glutamic acid with the aid of specific enzymes This process may be hindered by interference with the specific enzyme systems by lack of substrate or by anoxia Mechanical effects such as trauma pressure or oedema may block the delivery of substrate or oxygen to the critical areas of the brain (Fazekas and Bessman 1953)

In severe hypopituitarism the areas concerned with consciousness are unduly vulnerable and this may well be on account of changes within the neurones resulting from endocrine deficiency

Hornabrook (1956) studied the mechanism of coma in hypophysectomized rats using exposure to cold as the form of stress The results suggest that the sensitivity to cold after hypophysectomy may be divided into two phases the first immediate sensitivity which is apparent as soon as the animal recovers from operation and which soon disappears and the second a delayed and chronic sensitivity which does not develop until about 24 hours after operation and then increases in intensity for 2 or 3 weeks Adrenal cortical extract (Baird *et al* 1933 Tyslowitz and Astwood 1942) and thyroxine (Baird *et al* 1933) have both been shown to improve the resistance to cold of hypophysectomized rats Further Hornabrook (1956)

Infections

Acute infections such as colds influenza tonsillitis and gastro intestinal infection should be treated promptly with rest fluids salt the appropriate antibiotics and increased cortisone dosage These patients should be instructed to try to avoid contact with infections so far as possible

Surgery and anaesthesia

If possible general anaesthesia should be avoided and surgery restricted to emergencies When operation becomes inevitable it should be carried out under cortisone and it will probably be necessary to double or treble the dose The cortisone cover will have to be 100 milligrams a day or thereabouts and should be given 24 hours before operation and maintained for 4 days afterwards Cortisone should be used in operations on the pituitary gland whether or not there is prior evidence of hypopituitarism as it is possible that trauma or oedema in this region may block hypothalamic pituitary pathways through which the adrenal responses to stress are mediated The effectiveness of cortisone in the surgery of suprasellar tumours has been shown by Ingraham Matson and McLaurin (1952) Recent experiences of James (personal communication) have proved the effectiveness of cortisone before and after op ration on pituitary adenomas Since this regime has been introduced at the Dunedin Hospital 22 cases with pituitary adenoma have been operated upon without a death

Drugs

Hypnotics must be used sparingly and morphine omnopon pethidine and allied drugs not given Alcohol too should be avoided by these patients because of their decreased tolerance

Hypothermia

Sheehan and Summers (1952) demonstrated that on occasions elevation of the body temperature to normal could be achieved by the simple expedient of immersion in a warm bath They also emphasized some fallacies in the use of the clinical thermometer in these cases They measured the rectal temperature with a bacteriological thermometer and found temperatures there as low as 87 F (30 50 C) In these cases they noted depression of respiration and cardiac action and defective skin circulation not unlike the state of hibernation

Treatment of the coma

A therapeutic plan which covers most emergencies is as follows As soon as coma is discovered to be associated with hypopituitarism search should be made for a precipitating infection and the appropriate antibiotic measures commenced at once If the body temperature is found to be very low efforts should be made to raise it This should be done slowly over 30–60 minutes the patient being immersed in a warm bath and the temperature slowly elevated or a heat cradle used but carefully supervised The rectal temperature pulse rate and blood pressure should be checked at 5 minute intervals Blood should be taken for the estimation of sugar sodium potassium urea bicarbonate and the haematocrit Without waiting for the results and on the supposition that hypoglycaemia may be present glucose should then be given by mouth or by intravenous infusion

haemoconcentration suggests loss of sodium from adrenal failure in which case the sodium depletion in the blood may be masked by haemoconcentration Water intoxication is likely in a drowsy patient with low plasma sodium normal blood urea and normal blood pressure

TREATMENT

In view of the wide spectrum of precipitating factors in this syndrome of hypopituitarism with disturbances of consciousness and coma it is not surprising that there has been some divergence of opinion as to the optimum therapy in any particular case However from what has been said of the aetiology of these attacks in the foregoing sections the factor of a secondary adrenal failure has come into the foreground This appears to play a prominent part in the electrolyte disturbances the sodium depletion and in the bouts of hypoglycaemia and the hypersensitive insulin mechanism There are also the considerations of the hypothermia the secondary thyroid and gonadal failure and the factors of intercurrent infection and hypersensitivity to drugs and anaesthetics to be dealt with

Prior to the advent of cortisone the treatment of hypopituitarism of this type was by substitution with adrenal extracts and desoxycorticosterone thyroid substance testosterone or oestrogens This therapy often failed to protect patients from hypersomnia and hypopituitary coma Now and with recent progress in adrenal steroid therapy the way has been paved for the more effective management of this form of hypopituitarism and these patients can now be well protected In fact the arrival of cortisone has been a great benefit and it is now generally accepted that this is the most effective preparation for the treatment of these cases because of its having a combined electrolyte and glucocorticoid effect Prednisone and prednisolone on the other hand have a more selective glucocorticoid action but no sodium retaining powers and so are not so suitable for these cases They may promote fluid and sodium loss and cause muscle cramps

Earlier reports and trials with cortisone by Sheehan and Summers (1952) were not impressive in results but now most other workers have established clearly the value of cortisone in these cases and regard it as the mainstay of therapy Substitution treatment with end organ hormones is preferred to corticotrophin and stimulation therapy

Treatment of the syndrome

Oral cortisone 12 5–50 milligrams a day or the appropriate dosage as judged by results should be given to all patients with hypopituitarism who show signs of secondary adrenal failure with lack of water diuresis and a hypersensitive insulin mechanism with hypoglycaemia If there is hypothyroidism with an elevated plasma cholesterol or a lowered metabolic rate of more than minus 25 per cent a daily dose of *l* thyroxine 0 1–0 2 milligram or thyroid substance 1–2 grains (0 06–0 13 gramme) is also necessary Depot testosterone therapy (primotestone depot 250 milligrams monthly) promotes protein anabolism and improves sexual function in both males and females alike evidence of virilization in females may necessitate a change to oestrogens

With full substitution therapy in this manner many of these patients can be restored to a normal active life

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(5 per cent glucose saline solution) The quickest response to glucocorticoids is obtained by intravenous hydrocortisone (compound F) An infusion of 50–100 milligrams of hydrocortisone free alcohol (cortisol) dissolved in 250–500 millilitres of isotonic saline solution should be given as soon as possible Micro crystalline cortisone acetate can be used intravenously if hydrocortisone is not available Thereafter 50 milligrams of cortisone acetate should be given 6 hourly by nasal tube kept *in situ* for feeding until consciousness is restored

Further treatment must be regulated by the clinical and biochemical findings If hypoglycaemia is present intravenous or oral glucose should be continued The presence of hypotension haemoconcentration azotaemia and sodium depletion calls for intramuscular injection of 10 milligrams of desoxycorticosterone or fluorocortisol (from 0 25 to 1 milligram) and an adequate sodium intake It may be prudent to give 5 milligrams of desoxycorticosterone for 2–4 days as cortisone may initially increase the sodium output by elevation of a lowered glomerular filtration rate its sodium retaining function is inadequate to correct serious depletion of this ion If there is excessive water retention cortisone will correct this by allowing selective excretion of water in excess of sodium (Wynn and Garrod 1955) As soon as possible *l* thyroxine and testosterone should be added to the therapeutic plan

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CHAPTER 12

THE HORMONES OF THF SYMPATHETIC NERVOUS SYSTEM AND OF THE ADRENAL MEDULLA

H J C SWAN

INTRODUCTION

KNOWLEDGE and understanding in any branch of science usually advance in surges stimulated by the development of a necessary technique lack of which has prevented or inhibited progress The current interest in the physiology of the sympathetic nervous system and in the adrenal medulla—a notable feature of the biological advances of the last decade—has largely stemmed from the solution of two basic problems

The first of these was the identification and quantitative estimation of adrenaline and noradrenaline in tissue extracts and in body fluids (Euler 1946) Euler (1950) and Gaddum (1950) summarized the methods of assay extraction and purification used in their laboratories In spite of certain inherent limitations the biological method of quantitative estimation has found favour in many laboratories for tissue extracts and urine Lund (1950) and Weil Malherbe and Bone (1952) used a differential fluorometric method for estimation in blood plasma and for extracts of red blood cells

The second basic problem was solved when Tullar (1948) resolved *l* nor adrenaline from the racemic mixture of the *d* isomer and the *l* isomer thus pro viding in pure chemical form the necessary standard for comparative biological purposes Tullar (1949) also isolated in crystalline form *l* noradrenaline from extracts of adrenal glands giving evidence for its biological origin The nor adrenaline obtained from natural sources is uniformly the laevo isomer the dextro isomer not having been found in animal tissues and extracts Since the biological activity of the *d* isomer is only about 3 per cent of that of the *l* isomer the activity of the racemic mixture is about one half of that of the naturally occurring *l* isomer

As a result of these developments the nature of the humoral transmitter of sympathetic nerve effects has been more clearly established while certain more complex and more general effects of the sympathetic neurohormones are becoming apparent In this chapter the general aspects of the subject and those particularly pertinent to the human being are considered (For more detailed and specialized reviews *see* Euler 1950 1951 Goldenberg 1951 the *Symposium on Neuro humoral Transmission* 1954 and West 1955) This discussion is restricted to a consideration only of adrenaline (epinephrine) and noradrenaline (arterenol) as the effector substances of the sympathetic nervous system and of the adrenal medulla excluding the ganglion transmitter and centrally acting substances

THE SYMPATHETIC ADRENERGIC NERVE MEDIATOR

Transmitter substance of the sympathetic nerves

Stimulation of the sympathetic adrenergic nerve fibres besides producing a specific effect at an end organ also causes the liberation of a biologically active substance Because certain effects of this material resemble those of adrenaline the view was held that the transmitter of the sympathetic nerve impulse was adrenaline Similar effects were observed in testing extracts of nerves containing adrenergic nerve fibres The investigations of Cannon and Rosenblueth (1937) revealed certain differences between the action of adrenaline and the effects of sympathetic nerve stimulation which necessitated the elaboration of the theory of the sympathins to sustain the contention that adrenaline is the transmitter substance of the sympathetic nerves Although Bacq (1934) Greer *et al* (1938) and others pointed out that the action of noradrenaline resembled the effects of sympathetic nerve stimulation more closely than does the action of adrenaline the view persisted that adrenaline was the principal transmitter substance In the late 1940s improvements in the methods of extraction and biological assay of these substances and the availability of the laevo isomer of noradrenaline made possible the important conclusion that the principal active transmitter was nor adrenaline thus dispensing with the need for the sympathin theory

Noradrenaline

Because of the high probability that noradrenaline is the transmitter substance the occurrence of this substance in different parts of the body and in different species is of some importance (*see* Euler (1950) and West (1955)) Noradrenaline has been demonstrated in almost all the tissues in the mammal a notable exception being the placenta Logic and its demonstrated absence in the placenta (which lacks nerve fibres) prompted an investigation into the noradrenaline content of a number of different nerves in cattle (Euler 1946) It was demonstrated in the majority of structures examined but the concentration was greatest in those rich in adrenergic fibres the splenic nerves (8–18 micrograms per gramme) and the sympathetic trunk and the splanchnic nerves (2–4 9 micrograms per gramme)

Euler (1951) pointed out that the amount of noradrenaline recovered from splenic nerves increased from the proximal toward the distal portion To explain the total content of noradrenaline recovered from extracts of the spleen it is necessary to postulate that much higher concentrations exist at the terminal portions of the nerves than in the axons Euler (1956) suggested that noradrenaline was manufactured and stored in specific structural units in the axons whence it is continuously passed to the periphery of the nerve This storage would allow for the rapid repletion of transmitter substance which might be continuously released over long periods during stimulation

Section of the adrenergic nerves to the spleen kidneys and salivary glands in the sheep caused a reduction to near zero level of the adrenaline and noradrenaline content of these organs (Euler and Purkhold 1951) In the normal sheep heart Goodall (1951) found 0 15 microgram per gramme of adrenaline and 0 79 micro gram per gramme of noradrenaline Following complete cervicostellate ganglionec tomy the catechol content was reduced to values of 0 01 microgram per gramme of adrenaline and 0 13 microgram per gramme of noradrenaline The principal

effect resulted from removal of the right stellate ganglion There was a progressive rise in catechol amine content toward normal values over the following 6 weeks

Sympathetic denervation

Concerning the problem of sympathetic denervation there is the phenomenon of the return of tone to the peripheral blood vessels following sympathectomy in man and of the reactivity of these blood vessels to adrenaline and noradrenaline

Barcroft measured the rate of blood flow in the human hand or foot before and for some time after sympathectomy for hyperidrosis or other non arterial types of disease Although there was a great initial increase in flow in the dener vated limb (from 8 to 10 times control values) the flow progressively declined so that 1 week after sympathectomy the blood flow in the hand was about double and that in the foot about three times the pre operative value Further declines of flow took place in subsequent months Barcroft was impressed with the simi larity of the time course of the changes in blood flow with the development of hypersensitivity of certain biological preparations to adrenaline and noradrenaline which favoured the view that the development of a hypersensitivity to a circulating agent might be responsible for the recovery of tone by peripheral blood vessels (Barcroft and Swan 1953) Duff (1952) demonstrated hypersensitivity in the blood vessels of the hand to small doses of adrenaline given intra arterially as early as 6 days and as late as 1 year after sympathectomy The increase in sensitivity was such that the concentration of adrenaline necessary to produce an equal amount of vasoconstriction was four times as much in the normal as in the sympathecto mized hand In a later paper Duff (1955) compared the vasoconstrictor effects of adrenaline and noradrenaline before and after sympathectomy in patients who had Raynaud s disease A considerable increase in sensitivity was demon strated in about half of the hands studied and this was greater for adrenaline than for noradrenaline However vascular tone returned in all the hands including those in which hypersensitivity had not been demonstrated In view of the evidence for catechol amines in the circulation these observations are of significance in the mechanism of vasomotor control

Noradrenaline and adrenaline excretion rate

Both noradrenaline and adrenaline can be demonstrated in the urine Euler and Hellner (1951) have reported excretion values averaging respectively 29 0 and 11 5 micrograms per 24 hours in healthy subjects undergoing normal activity In another study Euler Hellner Bjorkman and Orwen (1955) noted that the excretion rate was much greater during the day than during the night Holmgren (1956) showed that after prolonged activity the rate of excretion of both adrenaline and noradrenaline was approximately doubled Assuming that urinary excretion represents 3 per cent of the total excretion rate it was calculated that 70 milli micrograms of noradrenaline were released into the circulation each minute Euler Luft and Sundin (1955) found that the excretion rate for both adrenaline and noradrenaline was increased threefold when the subject was tilted into the 45 degree upright position for a prolonged period

Euler Franksson and Hellstrom (1954) reported the excretion rate of nor adrenaline to be unaltered in patients following bilateral adrenalectomy and assumed that it had diffused from the sympathetic nerve endings to enter the

capillary or venous blood Euler and Luft (1951) had previously studied the excretion of noradrenaline during the intravenous infusion of this substance and found that about 3 per cent was recovered in the urine This has been used by Holmgren (1956) for the calculation of the rate of incretion into the circulation It does not necessarily follow that the daily urinary excretion of noradrenaline represents any fraction of similar magnitude of noradrenaline released at adrenergic nerve endings which would have to traverse the walls of blood vessels before entering the circulation Moreover it is probable that the enzyme systems near the adrenergic nerve endings are the more efficient inactivators of the transmitter substance Hence relatively large amounts of noradrenaline may be utilized daily in the mediation of the sympathetic nerve impulses The demonstration of cate chols in blood is more difficult but Weil Malherbe and Bone (1953) reported up to 5 micrograms of noradrenaline per litre of venous blood in resting subjects Correlations of blood levels and of urinary excretion rates may clarify the factors regulating the excretion of these substances

The formation and inactivation of noradrenaline has not been established with certainty (Blaschko 1954) Although most of the destruction takes place at the end organs some diffusion into the circulation appears to take place accounting for the constant quantity of catechols—principally noradrenaline—excreted in the free form in the urine Amine oxidase has been shown to inactivate both adrenaline and noradrenaline and it has been assumed to play this role in biological systems The observations of Euler and Zetterstrom (1955) and of Celander and Mellander (1955) indicate that this is probably not of major importance The former showed that the fraction of corbasil (unaffected by amine oxidase) excreted in the urine after its subcutaneous administration in man is about the same as that of adrenaline and noradrenaline Celander and Mellander (1955) compared the effects of adrenaline and noradrenaline so that under one circumstance the catechols passed directly to the test organs under the other they passed first through the vascular bed of the lower limbs They concluded that about 90 per cent were eliminated in a single passage through the vascular bed Their findings were similar even after the administration of inhibitors of the amine oxidase system

THE HORMONES OF THE ADRENAL MEDULLA

Although the adrenal medulla is part of the autonomic nervous system the nature of its hormones will be considered under a separate heading Its secretory cells are innervated by preganglionic fibres which correspond to the ganglion cell and postganglionic neurons of the autonomic system The emergency function of the secretory cells differs fundamentally from that of the sympathetic adrenergic fibres with their apparently continuous influence

For many years adrenaline alone was considered to be the active principle of the medulla Tullar (1949) and Euler and Hamberg (1949) demonstrated however that approximately 20 per cent of this vaso active material was in fact noradrenaline The presence of both hormones in the medulla may now be accepted as an estab lished fact the proportion of each amine present varies from species to species (West 1955)

Decline of hormone content

Interesting observations have been reported by Hokfelt (1951) and by West

et al (1953) on the change in the hormone content of the mammalian medulla with age West studied the organ of Zuckerkandl and the medulla in human necropsy material ranging from foetuses of 14 weeks gestation to adolescents aged 15 years Throughout gestation and for the first 2 months of life the proportion of noradrenaline present in both the adrenal gland and the organ of Zuckerkandl was almost 100 per cent From 2 months the proportion rapidly declined to 60 per cent at 1 year and to the fraction found in the adrenal medulla of the adult (20 per cent) at 3 years The authors suggested that during gestation and early life the noradrenaline from these sources served to maintain vasomotor tone until the development of the more differentiated autonomic nervous system was sufficiently complete to regulate the peripheral circulation

Medullary storage

Although the proportion of adrenaline and noradrenaline is relatively constant within an individual species it would be difficult to believe that a species specific equilibrium was obtained between the two compounds if they existed in the same cells (Euler 1951)

Detailed investigations of the medullary storage of adrenaline and noradrenaline were carried out by Hillarp and Hökfelt (1953) Hillarp *et al* (1953) and Blaschko *et al* (1955) The vaso active material occurs within specific granules contained in the medullary cell which behave as simple osmotic systems the catechols are normally unable to pass from within the granule Nevertheless the rapid release of the stored catechols on rupture of the granules suggested that they existed in a soluble and active form within its structure Hillarp and Hokfelt suggested that while all cells can form noradrenaline only certain specific cells can methylate noradrenaline to adrenaline Evidence supporting the contention that cells exist which are specific for either adrenaline or noradrenaline was provided by Folkow and Euler (1954) who demonstrated that stimulation of the different areas of the hypothalamus can alter the quantity of adrenaline or noradrenaline in the supra renal vein blood These observations also indicate the presence of specific centres in the central nervous system and efferent pathways to separate storage depots in the adrenal gland for each of the catechols

Glandular release under stimulation and stress

The outflow of adrenaline and noradrenaline from the adrenal gland has been studied by Bulbring and Burn (1949) who showed that both catechols were present after stimulation of the splanchic nerves in the cat Kaindl and Euler (1951) demonstrated that the output of both catechols was increased to about four times the resting values during a period of bilateral carotid occlusion and that nor adrenaline predominated in the mixture

Hillarp and Hökfelt (1953) and Euler (1951) expressed the opinion that nor adrenaline was an independent separate hormone of the adrenal medulla Under conditions of severe circulatory stress such as asphyxia noradrenaline is the pre dominant vaso active substance released from the gland Under other circum stances for example hypoglycaemia (Duner 1953) adrenaline is principally released *The experiments of* De Largy *et al* (1950) *indicate that* in the human subject even when equal amounts of adrenaline and noradrenaline are present

in a mixture the effects resemble those of adrenaline more closely than those of noradrenaline

THE EFFECTS OF ADRENALINE AND NORADRENALINE IN MAN

In cardiovascular physiology the pressor effect of adrenaline is frequently demonstrated It is probable that this acute and severe hypertension if it occurs at all is *an infrequent phenomenon in normal biology* Such reactions are of limited value in determining the role which adrenaline plays in the economy of living creatures unless certain criteria have been met The dose of vaso active material must have a relation to the biological values The route and the instantaneous or continuous nature of the injection must be specified The concentration value arriving at the effector organ following a sudden discontinuous intravenous injection is frequently out of all proportion to that which might occur physiologically The status of the animal preparation may well be the most important determinant of a response not only from quantitative but also from qualitative aspects

The effect of adrenaline and noradrenaline on the human circulation was reported on by Goldenberg *et al* (1948) and by Barcroft and Swan (1953)

Subjective effects

An early sensation with continuous intravenous infusion of 10 micrograms of adrenaline per minute was a feeling of expectancy with a mild tingling over the body Within 10 seconds there was an increase in depth and rate of respiration and subjective symptoms were associated with hyperventilation At this period it was possible to hold a moderate inspiration for only 10–15 seconds About 10 seconds after the start of the respiratory symptoms the heart rate increased this was not always noticed by the subject Gradually the force of the beat increased for a further half minute after which the rate frequently fell to within a few beats of its resting value The subject was now aware of a forceful character to the heart beat Shortly after the start of the subjective effects on the heart there was a feeling of fatigue which usually started in the back and spread into both legs There was a coarse tremor of the hands and feet After the infusion had been continued for periods of greater than 3 minutes the symptoms diminished very considerably

With noradrenaline (10 micrograms per minute) the symptoms differed in that the feeling of anxiety and expectancy was absent There was an increase in respiration but not to the same extent as with adrenaline and palpitation fatigue and muscle tremor were absent In contrast to the symptoms accompanying adrenaline infusion those caused by noradrenaline were never familiar to the subject

Objective effects

Differences were observed in the changes in arterial blood pressure heart rate cardiac output and peripheral resistance caused by each substance With adrenaline the mean arterial blood pressure showed little or no change Following a brief

initial decline there was an increase in systolic pressure The diastolic pressure declined significantly In contrast to this noradrenaline caused an increase in the systolic the mean and the diastolic pressures during the period of infusion Adrenaline caused a brisk tachycardia lasting for about 30 seconds after which the heart rate gradually fell to about 10 beats per minute above the resting value With noradrenaline the heart rate always slowed 10-30 beats below the control level This slowing could be abolished by atropine and is presumably a vagal reflex in response to the elevation of arterial blood pressure The change in cardiac output caused by adrenaline and noradrenaline was measured by Goldenberg *et al* (1948) using the Fick method and by Barcroft and Starr (1951) who used the ballistocardiograph These authors concluded that whereas adrenaline increases cardiac output with noradrenaline it is either unchanged or decreased As a consequence of the increase in cardiac output associated with minimal change in mean blood pressure it can be concluded that within the dose range used and in the normal human subject adrenaline acts as an over all vasodilator At the same dose level noradrenaline causes a minimal change in cardiac output and a considerable increase in mean blood pressure indicating an over all vasoconstrictor action

Local blood flow

In an attempt to define the location of the changes in peripheral vascular resistance the rate of blood flow in different parts of the body has been determined by several investigators (Barcroft and Swan 1953) In the normal subject the blood flow through the liver kidneys skeletal muscle and brain accounts for the greater part of the cardiac output The values for the blood flow in different organs (Table I) afford independent evidence consistent with the conclusions as to the changes in peripheral resistance calculated from data on cardiac output and peripheral resistance The over all vasodilator effect of adrenaline is essentially due to an increase in blood flow through the liver and skeletal muscles In the case of noradrenaline the unchanged or decreased nature of the flow associated with the rise in mean arterial blood pressure indicates a considerable increase in total peripheral resistance

TABLE I

EFFECT OF ADRENALINE AND NORADRENALINE ON THE BLOOD FLOW THROUGH INDIVIDUAL ORGANS

Organ	*Adrenaline*	*Noradrenaline*
Liver	100 increase	No material effect
Kidney	40 decrease	20 decrease
Skeletal muscle	100 increase	No effect or actual decrease
Brain	20 increase	Small decrease

Pulmonary arterial pressure

Fowler *et al* (1951) showed that the pulmonary arterial pressure was increased during infusion of noradrenaline However since the pulmonary capillary (or

wedge) pressure was equally elevated it was concluded that noradrenaline in the dosage used did not significantly elevate the pulmonary arteriolar resistance

Differences in action on the metabolic processes

Differences also exist between the action of the two substances on the metabolic processes (Euler 1951) In amounts of the order stated previously adrenaline is from 5 to 10 times as active as noradrenaline in regard to oxygen consumption (Goldenberg *et al* 1948) to release of glucose from the liver and to specific inhibition or stimulation in the nervous system The effectiveness of noradrenaline in reducing the circulating eosinophils was only one sixth that of adrenaline (Humphreys and Raab 1950)

The role of adrenaline and noradrenaline

These observations on the intact unanaesthetized human subject accord well with the view that noradrenaline is the principal adrenergic nerve transmitter In quantities sufficient to cause a sustained and not inconsiderable rise in blood pressure its metabolic effects are not those of an emergency substance In contrast the effects of adrenaline a hormone with a general action away from the site of its liberation are those which might characterize an emergency substance Indeed the subjective effects which accompany intravenous infusions of adrenaline are frequently familiar to the subjects of such experiments having been experienced previously under conditions of emotional or physical stress Although the outflow from the adrenal vein has been shown to contain a proportion of noradrenaline in the human subject the subjective metabolic and circulatory effects of adrenaline predominate in mixtures containing equal parts of each substance

In conclusion it may be stated that the effects of biologically reasonable quantities of adrenaline and noradrenaline as revealed by these studies on the human subject do much to indicate the correctness of the assumptions of Cannon and Rosenblueth (1937) in regard to the role of adrenaline and of the adrenal medulla in normal physiology

RELATION OF ADRENALINE AND NORADRENALINE TO PATHOLOGICAL STATES

Essential hypertension

In hypertension from most causes the cardiac output is within the normal range The derangement of function therefore is due to an increased vascular resistance which is largely due to a reduction in the calibre of the small blood vessels That this reduction is initially related to a vasospastic condition is evident because of the vasodilatation that these vessels undergo when the influence of the sympathetic nervous system is abolished or reduced by the specific central vasomotor depressant agents by ganglion blocking agents or by surgical sympathectomy Since noradrenaline has been demonstrated to be the transmitter substance of the adrenergic portion of the sympathetic nervous system its possible implication in the genesis of hypertension is evident Increased production of noradrenaline at the nerve endings supersensitivity of the blood vessels to the mediator or impairment of its

destruction could result in a reduction in the calibre of these vessels an increase in the total peripheral resistance and resulting hypertension Indeed Goldenberg *et al* (1948) suggested that essential hypertension might be due to defective trans methylation of noradrenaline to adrenaline but they were careful to point out that there was no definite evidence for this view Judson *et al* (1950) had no evidence to suggest that patients with hypertension were more sensitive than normal subjects to the pressor effects of single intravenous doses of adrenaline or noradrenaline Since the plasma concentration values (Manger *et al* 1954) and the urinary excretion rate (Euler Hellner and Purkhold 1954) of noradrenaline in such patients do not significantly exceed the values observed in normal subjects excessive production of noradrenaline at the nerve endings is unlikely The virtual abandonment of surgical sympathectomy in favour of the use of central vasomotor depressant drugs with ganglion blocking action in the treatment of clinical hypertension and the phenomenon of the return of vascular tone to certain normal blood vessels in subjects after sympathectomy indicate that local processes within the muscle cells of the small arteries and arterioles are fundamentally con cerned in establishing the balance between the tension in the vessel wall and the distending force within these vessels The factors determining the level at which this balance is set are poorly understood and the question of whether the sympa thetic transmitter is concerned directly or indirectly in the determination of this balance is at present unanswered

Phaeochromocytoma

In contrast to the lack of data to associate adrenaline and noradrenaline with essential hypertension there is ample evidence to relate these substances to the secretory tumours of the adrenal medulla The presence of both adrenaline and noradrenaline in extracts of tumours removed at operation was first demonstrated by Holton (1949) since when these amines have been repeatedly found Golden berg *et al* (1950) reported that the proportion of noradrenaline ranged from 14 to 90 per cent in 10 tumours of weights from 21 to 567 grammes The concentration varied between 0 03 and 7 65 micrograms per gramme for adrenaline and between 1 02 and 6 96 micrograms per gramme for noradrenaline Goldenberg pointed out that the differences seen in normal subjects between the effects of small doses of adrenaline and noradrenaline no longer applied in such patients because of the high rate of release of the amines into the circulation Under this circumstance noradrenaline appears to have metabolic in addition to pressor effects while adrenaline causes an over all increase in peripheral resistance as well as an increase in metabolism

Lund (1952) in a case of phaeochromocytoma demonstrated a significant elevation in the plasma concentration of adrenaline and noradrenaline and in the rate of urinary excretion Manger *et al* (1954) reported on the concentration of noradrenaline and adrenaline in the plasma of 13 patients with phaeochromo cytoma *In each of 9 patients with sustained hypertension* concentration of pressor amines was elevated to levels greater than 12 micrograms per litre of plasma for the sum of adrenaline and noradrenaline The latter was the predominant amine present in most instances In 2 of the 4 remaining patients in whom the arterial hypertension was paroxysmal the plasma concentration was normal but significantly elevated values were obtained after administration of a provocative drug for example histamine

CONCLUSIONS

Space does not permit a more complete discussion of the role of adrenaline and noradrenaline in the mechanism of the hypoglycaemic response or of the relation ship of these substances to the actions of the thyroid or adrenal cortical hormones It is apparent however that noradrenaline and adrenaline may exert a wide range of influence in endocrine activity

In the last decade the advances in knowledge of the physiology of the hormones of the sympathetic nervous system and adrenal medulla have been more basic than applied As a result principally of the work of Euler the nature of the transmitter substance has been established and the role of noradrenaline in normal biological processes clarified The presence of both adrenaline and noradrenaline in the outflow from the adrenal medulla has been taken to indicate a dual function of medullary secretion The predominant role of adrenaline as an emergency sub stance has been sustained In pathological states except phaeochromocytoma the role of noradrenaline and adrenaline has still to be elucidated

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CHAPTER 13

ADRENAL FUNCTION TESTS

C L COPE

THE WATER EXCRETION TEST

THE ABILITY of the kidney to produce a water diuresis is greatly impaired in states of adrenal deficiency The phenomenon has been utilized in the test developed by Robinson Power and Kepler (1941) which is widely used The excretion defect can be completely restored by cortisone or hydrocortisone and the prompt manner in which this occurs shows that the defect is due to shortage of the steroid

A simplified water excretion test was first proposed by Soffer and Gabrilove (1952) The phenomenon of restoration of diuretic function by cortisone has been studied in detail by Garrod and Burston (1952) They showed that it occurs 2½ hours after 50 milligrams of cortisone acetate have been administered orally and that maximum diuresis occurs at about 5 hours The effect which persists for about 12 hours but has ceased in 18–24 hours is specific for cortisone or hydro cortisone and is not shown for instance by testosterone or desoxycorticosterone The ability to improve poor diuretic power in this way in a given case is strong evidence that the patient is suffering from adrenal deficiency

As a screening test for Addison s disease or other forms of adrenal insufficiency the water excretion test is of very real value having among its advantages the great merit of simplicity

The water excretion test is best carried out in the forenoon The bladder is first emptied and then a dose of water equivalent to 20 millilitres per kilogram body weight and slightly warmed is drunk steadily over a period of 30–45 minutes the quantity is not critical Patients should be kept at rest during the test and urine collections made at hourly intervals for 4 hours The volumes of these urine samples are measured and the total output in 4 hours recorded as a percentage of the ingested water volume In normal subjects 50 per cent or more will usually be excreted in the first 2 hours with a total of at least 80 per cent during 4 hours

In adrenal insufficiency the total eliminated in 4 hours may be less than 20 per cent and an excretion of less than 50 per cent is suggestive of adrenal deficiency However a number of other clinical disorders may impair water diuresis These can usually be excluded by routine clinical observation so that confusion will not often occur Such conditions include renal failure congestive cardiac failure gross oedema ascites severe obesity and unaccustomed cigarette smoking

If the 4 hour excretion exceeds 50 per cent the test should be regarded as negative If it is less than this an attempt should be made with cortisone to restore normal diuresis For this purpose the water test is repeated with an oral dose of 50 milligrams of cortisone acetate given 4 hours before the water Occasionally this dose proves insufficient and 100 milligrams may then be tried

Performed in this way the test is a valuable indicator of adrenal deficiency and now supplants the more complex test of Robinson Power and Kepler It does not distinguish between primary (addisonian) and secondary (hypopituitary) causes of adrenal deficiency This distinction could be made by repeating the water test after an effective dose of corticotrophin which should restore some diuretic power if the defect is due to hypopituitarism However in practice it is usually better to employ other methods for making this distinction

EOSINOPHIL DEPRESSION TESTS

That the eosinophil cells present in circulating blood tend to disappear under the influence of cortisone or cortisol was first shown by Hills Forsham and Finch (1948) They further showed that there was a prompt fall in the eosinophils after corticotrophin but that no such fall occurred in subjects with Addison s disease The fall is a direct result of the rise in the concentration of hydrocortisone in the blood and the magnitude of the eosinophil fall is very roughly proportional to the hydrocortisone rise

The cells are easily counted by suitable methods and it is not necessary to do a complete differential count Special stains are used which cause the lysis of red cells and of most white cells leaving unchanged only the much more resistant eosinophils Several solutions are available the best known being the acetone eosin stain of Dunger (1942) and the phloxine propylene glycol stain of Randolph (1944) The main advantage of the former is its simplicity and speed but it is subject to several serious errors and has been severely criticized by a number of investigators It causes some lysis of the eosinophil cells and also evaporates quickly so that agility is required for its effective use The author does not use it in his own laboratory The Randolph stain is more preferable and though it takes a little longer is much more reliable since the cells are more stable in this solution This stain does not evaporate quickly under the microscope and the cells are easily seen From a considerable experience of eosinophil counting the Randolph method has proved the most reliable in our hands

Randolph stain—A freshly made mixture of equal parts of 0 1 per cent phloxine and 0 1 per cent methylene blue (microscopic stain) each dissolved separately in 50 per cent aqueous propylene glycol Blood is drawn to the 0 5 mark on a white cell pipette and diluted to the top mark with the diluting fluid The whole is well shaken Counting is best done in a Fuchs Rosenthal chamber after this has been allowed to stand for 10 minutes or more to allow the cells to stain and to settle

Normal eosinophil counts vary between 60 and 500 per cubic millimetre It is not the absolute count but the percentage drop from the initial level which is the significant figure in any test based on these cells

Depression by stress

Circulating eosinophils decrease after a wide variety of noxious procedures (for a review of the literature *see* Hills Forsham and Finch 1948) The major cause of this drop seems to be the adrenal stimulation which results from such influences Infections accidents fractures surgical operations coronary thrombosis all tend to be followed by a fall in eosinophil cells lasting from 3 to 5 days Such a fall provides indirect evidence of a healthy response of the adrenal cortex but if no fall occurs an inadequately responding adrenal cortex is to be suspected Several cases have been recorded in which a lack of eosinophil drop during an

unsatisfactory convalescence gave the first clue to the discovery of an unsuspected Addison s disease The post operative eosinophil count is a simple test which has a useful screening value in any post operative or post traumatic condition in which for any reason such as excessive shock adrenal inadequacy may be suspected

Response to adrenaline

For a short period in the early studies of the eosinophil response it was believed that adrenaline caused adrenal cortical stimulation since its injection caused a prompt fall in eosinophil cells Observation of the eosinophil response to injected adrenaline was therefore proposed as a test of adrenal function by Recant *et al* (1948) They claimed that while persons with normal adrenals gave a drop of 70–80 per cent after 4 hours the drop in Addison s disease was much less In the following year Thorn and Forsham (1949) concluded that a fall of more than 50 per cent after adrenaline excluded serious cortical deficiency Unfortunately the experience of others has not been able to confirm the utility of the test the author early obtained large eosinophil drops in persons with complete hypo *pituitarism and negligible adrenal activity Organized criticism came at first from* French and Swiss workers Mathieu de Fossey and Detour (1950) found that adrenaline often gave a 60 per cent drop in eosinophils in undoubted Addison s disease and Ruppel and Hitzelberger (1951) noted that adrenaline caused a fall without the intervention of the adrenal cortex A progressively widening experience revealed the unreliability of the test using adrenaline as a stimulus Best Muehrcke and Kark (1952) after a very careful appraisal concluded that the diagnostic value of negative responses to adrenaline was practically nil

The test is often misleading and should never be used

Depression after corticotrophin

In contrast to these criticisms of adrenaline as a test stimulus the value and specificity of corticotrophin is thoroughly established but details of technique especially the mode of administration have an important influence on reliability

With efficient stimulation there is a rapid rise in the hydrocortisone output of the adrenal cortex but some lag in the fall in eosinophil cells These have usually reached their lowest level in 4 hours With soluble corticotrophin administered intramuscularly hydrocortisone output is usually maximal in about 1 hour but after intramuscular gel the peak of steroid excretion is not reached until 3–4 hours For the eosinophil depression test therefore either intramuscular or intravenous soluble corticotrophin should be used Jenkins *et al* (1955) have made a valuable comparison of the diagnostic use of these two methods in Addison s disease

The rapid corticotrophin test

A rough screening test can be made by using intramuscular corticotrophin A count is first made of the circulating eosinophils and 25 milligrams of soluble corticotrophin then injected intramuscularly Four hours later a second count is made the fall being expressed as a percentage change from the original level A drop in eosinophils of over 50 per cent is evidence of good adrenal function but a smaller drop cannot safely be regarded as indicating adrenal inadequacy It does however indicate the need for further investigation

Jenkins *et al* (1955) have extensively investigated this test In a series of over 500 tests eosinophil drops of less than the arbitrary norm of 50 per cent were encountered in 24 per cent of the normal controls The drop in eosinophils in persons with intact adrenals ranged from 0 to 100 per cent whereas the response in Addison s disease lay between a 72 per cent rise and a 69 per cent fall With this test therefore there is considerable overlap between the two groups and ambiguous results will often be encountered It remains nevertheless a test with value for screening purposes

A big factor contributing to these inconsistent results is believed to be irregular absorption from the injection site This can be avoided by giving the corticotrophin as a continuous intravenous drip Much better results are then obtained

The intravenous corticotrophin test

In this test an initial eosinophil count is made and then a slow intravenous drip of 25 milligrams of corticotrophin started and continued for about 8 hours The second eosinophil count is made at the termination of the infusion The corticotrophin is dissolved in one pint of normal saline solution to provide sufficient volume of vehicle It is important that saline solution and not 5 per cent glucose should be used as the vehicle the author has recently seen a full addisonian crisis provoked by the inadvertent use of the latter vehicle

With this technique ambiguous results should be rare since no overlap between Addison s disease and normal groups occurs In a large series studied by Jenkins *et al* (1955) eosinophil depression in subjects with normal adrenals was 75–100 per cent with a mean of 94 per cent Eosinophil depression in subjects with Addison s disease ranged from an increase of 70 per cent to a fall of 50 per cent the mean for this group being a 1 per cent fall only

This intravenous corticotrophin test is the most sensitive and reliable that we have at present for the detection of adrenal cortical deficiency it should be used as the final arbiter in all doubtful cases

17 KETOSTEROID EXCRETION

For the estimation of the 17 ketosteroids it is desirable that an approved procedure such as that recommended by the Medical Research Council Committee (1951) should be adhered to Using such a method the normal range for male adults is from 8 to 26 milligrams daily (mean 13 milligrams) and for women from 5 to 17 milligrams (mean 8 milligrams) the higher figures in the male being due to a proportion of the ketosteroids derived from androgens produced by the testes (Hamburger 1948)

Factors influencing output

Since most of the 17 ketosteroid is derived from adrenal cortical hormones a complete cessation of adrenal function will result in a fall to zero of the output in the female and a fall to below 5 milligrams per day in the male There are however other causes of a reduction in the 17 ketosteroid output and a low output is not proof though it is suggestive of adrenal deficiency It indicates the need for more definitive investigation

If the 17 ketosteroid output is normal or raised Addison s disease or adrenal deficiency due to hypopituitarism can be excluded provided no exogenous source of ketosteroid such as cortisone or other steroid therapy is being given at the time Among the influences which may lead to a low output are the extremes of age Under 5 years the output averages normally only 1 4 milligrams daily and between 5 and 12 years the mean output is 3 8 milligrams With puberty there is a rapid increase in output the normal adult figures being attained in about 3 years Over the age of 50 years there is a steady decline in the output to half or less of the normal adult figure (Kenigsberg Pearson and McGavack 1949)

Severe debilitating states

Almost any chronic illness can lead to a reduced output and in severe debilitating states it may be particularly low A review of the ketosteroid output in such disorders has been made by Forbes *et al* (1947) The mean output in 82 males with chronic disease was 6 2 milligrams per day and in 114 females 4 4 milligrams per day

In severe debilitating states such as malignant disease or tuberculosis weakness wasting and low blood pressure are often associated with hypochloraemia and a tendency to pigmentation and a suspicion of Addison s disease may arise The finding of a low 17 ketosteroid output will increase this suspicion but in such cases the intravenous corticotrophin test is desirable for the confirmation of adrenal disease

High adrenal activity

Although low adrenal activity is always associated with low 17 ketosteroid output unless exogenous hormone is being given high adrenal activity is not necessarily associated with a high 17 ketosteroid output This will depend on whether excessive androgen production is taking place In certain cases of adrenal adenoma or carcinoma and less frequently hyperplasia such an over production occurs with resultant high 17 ketosteroid output In the female there may be clinical evidence of virilization but in typical Cushing s syndrome the 17 keto steroid output is only slightly above or even below the normal mean the over production being mainly of hydrocortisone

Ketosteroid response to corticotrophin

Use of the 17 ketosteroid output as an index of adrenal stimulation by intra venous corticotrophin has not been justified by results A rise in output is not certainly provoked even in normal subjects Jenkins *et al* (1955) compared the effectiveness of this test with that of the eosinophil depression after corticotrophin In 41 tests in patients with Addison s disease the mean rise was 0 5 milligram daily In contrast 151 tests were performed in control subjects and these showed a mean rise of 5 milligrams daily Since the normal control range of response completely overlapped the range of response in Addison s disease ambiguous results were frequent

The use of this test therefore is not justified when the results with eosinophil depression are so satisfactory

Jenkins *et al* (1955) have extensively investigated this test In a series of over 500 tests eosinophil drops of less than the arbitrary norm of 50 per cent were encountered in 24 per cent of the normal controls The drop in eosinophils in persons with intact adrenals ranged from 0 to 100 per cent whereas the response in Addison s disease lay between a 72 per cent rise and a 69 per cent fall With this test therefore there is considerable overlap between the two groups and ambiguous results will often be encountered It remains nevertheless a test with value for screening purposes

A big factor contributing to these inconsistent results is believed to be irregular absorption from the injection site This can be avoided by giving the corticotrophin as a continuous intravenous drip Much better results are then obtained

The intravenous corticotrophin test

In this test an initial eosinophil count is made and then a slow intravenous drip of 25 milligrams of corticotrophin started and continued for about 8 hours The second eosinophil count is made at the termination of the infusion The corticotrophin is dissolved in one pint of normal saline solution to provide sufficient volume of vehicle It is important that saline solution and not 5 per cent glucose should be used as the vehicle the author has recently seen a full addisonian crisis provoked by the in advertent use of the latter vehicle

With this technique ambiguous results should be rare since no overlap between Addison s disease and normal groups occurs In a large series studied by Jenkins *et al* (1955) eosinophil depression in subjects with normal adrenals was 75–100 per cent with a mean of 94 per cent Eosinophil depression in subjects with Addison s disease ranged from an increase of 70 per cent to a fall of 50 per cent the mean for this group being a 1 per cent fall only

This intravenous corticotrophin test is the most sensitive and reliable that we have at present for the detection of adrenal cortical deficiency it should be used as the final arbiter in all doubtful cases

17 KETOSTEROID EXCRETION

For the estimation of the 17 ketosteroids it is desirable that an approved procedure such as that recommended by the Medical Research Council Committee (1951) should be adhered to Using such a method the normal range for male adults is from 8 to 26 milligrams daily (mean 13 milligrams) and for women from 5 to 17 milligrams (mean 8 milligrams) the higher figures in the male being due to a proportion of the ketosteroids derived from androgens produced by the testes (Hamburger 1948)

Factors influencing output

Since most of the 17 ketosteroid is derived from adrenal cortical hormones a complete cessation of adrenal function will result in a fall to zero of the output in the female and a fall to below 5 milligrams per day in the male There are however other causes of a reduction in the 17 ketosteroid output and a low output is not proof though it is suggestive of adrenal deficiency It indicates the need for more definitive investigation

hydroxyl groups attached to the 17 and 21 carbon atoms and a ketone group at the 20 carbon atom

For this group of 17 21 dihydroxy 20 ketosteroids a fairly specific colour reaction was discovered by Porter and Silber (1950) This reaction is based on the development of a yellow colour when solutions containing the steroid are allowed to react with phenylhydrazine and sulphuric acid The reaction is a sensitive one and with pure solutions of steroid gives a linear relation between colour and concentration down to dilutions as low as 1 microgram per millilitre The group of steroids which can be estimated by this reaction have come to be called the corticosteroids or 17 hydroxy corticosteroids Corticosteroid assay can be made in either plasma or urine but in each case it is necessary to submit extracts to a preliminary purification to remove interfering chromogens

Plasma concentrations

In plasma the amount of these steroids is very small Very considerable care practice and technical skill are needed if reliable results are to be obtained because of the minute quantities involved also chromatographic purification of the plasma extracts is necessary before applying the colour reaction While the estimation of plasma corticosteroids has been of great research value it is not really suitable for routine clinical use except in specialized laboratories It is doubtful whether the effort is justified by the clinical information yielded

The normal range of values is from 3 to 20 micrograms per 100 millilitres the former figure being at the lower limit of sensitivity of the method Considerable fluctuations occur during the day and in response to activity (Bayliss 1955) The plasma concentrations found in Addison s disease are low but not completely zero so that they may frequently overlap the lower normal limits Conversely in Cushing s syndrome the plasma corticosteroid levels are frequently above the higher normal limit but by no means invariably so As a consequence figures within the normal range may be encountered in both diseases and results are therefore sometimes difficult to interpret In cases which are clinically ambiguous the plasma corticosteroid results are likely to be ambiguous also Peterson and Wyngaarden (1956) have obtained evidence from isotope studies which suggests that changes in production or utilization of hydrocortisone are not necessarily shown in the plasma concentrations

In spite of these objections a simple plasma corticosteroid method would probably be of clinical value Silber and Porter (1954) adapted their colour reaction for use as a plasma estimation and Wallace Christy and Jailer (1955) reported on its clinical use They found in normal patients a range of 4–39 micrograms per 100 millilitres and the same range in non endocrine hospital subjects In 9 cases of Cushing s syndrome values were mostly between 34 and 57 micrograms the highest being 107 micrograms but 2 fell within the normal range These authors considered that the plasma levels reflected adrenal hyperfunction more accurately than did urinary steroid excretion Much depends however on what steroid fraction is estimated in the urine

Urinary corticosteroid excretion

The application of the relatively simple Porter Silber colour reaction for the determination of the corticosteroid content of urine has attracted many workers

KETOGENIC STEROIDS

Norymberski Stubbs and West (1953) introduced a new chemical method for the study of adrenal cortical metabolism Steroids produced by the adrenal cortex have a short side chain attached at the 17 carbon atom This side chain can be oxidized away by periodic acid with liberation of formaldehyde or acetaldehyde this can be measured giving a figure for the formaldehydogenic steroids The residual steroid is a 17 ketosteroid which can be estimated by the usual method for this group of compounds Brooks and Norymberski (1953) found that sodium bismuthate is a more satisfactory oxidizing agent for the adrenal group of steroids

If then in a urine extract the 17 ketosteroids are estimated by the usual method both before and after oxidation with sodium bismuthate the increase in the second estimation will give the quantity of steroid produced by the oxidation that is the 17 ketogenic steroid This procedure is chemically a specific one and thus estimates a group of steroid metabolites the greater part if not all of which are derived from the adrenal cortex By this technique known adrenal steroids added to urine can be detected in an 85–95 per cent yield In support of the reliability of the method Norymberski Stubbs and West (1953) found very low figures in Simmonds disease and in a patient with Addison s disease Corticotrophin caused a rise in output though of variable degree

Excretion range

The normal range of excretion of 17 ketogenic steroids lay between 9 6 and 19 2 milligrams daily for men and 4 6 and 13 4 milligrams daily for women Other workers have found essentially similar figures Thus Diczfalusy *et al* (1955) found a range of 11–22 milligrams in 17 normal men and 6 9–17 9 milligrams in 20 normal women the respective means being 15 and 10 6 milligrams Schuller (1956) in a much larger survey found the same mean figures but in normal subjects a rather wider range attaining to 36 milligrams in males and 28 milligrams in women Schuller also studied the effects of age finding excretion maximal in both sexes between 20 and 55 years

The value of this test in diagnosis is not yet adequately defined as sufficient work has not been done on the variations in disease states Several workers have reported low figures in hypopituitarism and in Addison s disease but equally low figures have been found in subjects in whom the adrenals were not suspect Both high and normal values have been found in clinically certain Cushing s syndrome *Such ambiguities are to some extent inevitable in any measurement* based on an unstimulated adrenal for the normal adrenal may sink to a degree of inactivity comparable to that encountered in Addison s disease On the other hand activity in Cushing s syndrome may be subject to wide daily fluctuations being on some occasions within normal limits (Birke Plantin and Diczfalusy 1956) The test has been studied by Levell *et al* (1957) and by Moxham and Nabarro (1956)

TESTS BASED ON CORTICOSTEROID ASSAY

The adrenal cortical steroids are characterized by the possession of a short side chain attached to the 17 carbon atom of the molecule and in many there are

Virilizing states

The same phenomenon can be used for diagnostic purposes with virilizing adrenal cortical lesions with a raised ketosteroid output Those due to adrenal hyperplasia can be inhibited by cortisone whereas those due to tumour are resistant to such inhibition The test consists in observing for 2–5 days the effect of oral or intra muscular cortisone on the urinary 17 ketosteroid excretion In general a dose of 200 milligrams daily is sufficient Jailer *et al* (1954) applied this test to 36 patients with adrenal cortical over action Reduction in 17 ketosteroid excretion occurred in all the 14 female pseudo hermaphrodites with adrenal hyperplasia and in 6 boys with macrogenitosomia praecox and adrenal hyperplasia Five subjects with virilism due to adrenal tumour were investigated but in none was there inhibition Segaloff Gordon and Horwitt (1955) showed that inhibition occurs in 4–6 hours after intravenous infusion of hydrocortisone

Cushing s syndrome

Jailer *et al* (1954) applied this cortisone test to Cushing s syndrome Five cases of adrenal adenoma or carcinoma showed no fall in 17 ketosteroid excretion after cortisone 1 of 6 cases of adrenal hyperplasia showed no inhibition and in another the inhibition was only slight The response therefore appears to be less clear cut in Cushing s than in the virilizing syndromes This may be due to the fact that exogenous cortisone can partly metabolize to 17 ketosteroid so that the effects of inhibition of endogenous cortisone may be masked by the cortisone administered These difficulties in Cushing s syndrome can probably be overcome by using a different suppressing agent For this purpose the very potent substituted steroid 9 α fluorohydrocortisone has proved of value and Cope (1956) found that with this substance hydrocortisone excretion in the urine can be completely inhibited in the normal case and in Cushing s syndrome due to hyperplasia For clinical purposes in Cushing s syndrome the demonstration of reduced excretion of keto genic steroids is a better criterion of inhibition than the study of 17 ketosteroid excretion A dose of 10 milligrams daily by mouth of 9 α fluorohydrocortisone will produce full inhibition in sensitive subjects and this should be achieved by the third day

ALDOSTERONE

The clarification of the clinical syndrome of primary aldosteronism by Conn (1955) has led to a large demand for tests for excessive aldosterone production in man Two assay methods are available for aldosterone in urine The first is a bio assay based on alteration in the sodium potassium ratio in the urine of adrena lectomized rats after injection of extracts containing aldosterone (Simpson and Tait 1952 Llaurado 1956) but the technique is difficult and time consuming In laboratories accustomed to animal assay work it should be possible to determine whether a given sample has a high normal or low aldosterone content but a greater degree of accuracy than this is difficult to achieve without considerable extra labour

The second method for aldosterone assay in urine is physicochemical and has been introduced by Neher and Wettstein (1956) This is also a laborious technique and depends on the separation of aldosterone by two successive developments of

but there are many difficulties First the concentration of free steroid in urine is small amounting to only 100–300 micrograms per day Secondly many non specific chromogens exist in urine which interfere seriously with the measurement of the specific yellow colour Thirdly in unpurified urine circumstances exist which may actually lead to a negative value for the apparent steroid content even when normal quantities are known to be present If urinary extracts are purified by paper chromatography fair estimates of the adrenal steroid content can be made by the Porter Silber reaction (Cope and Hurlock 1952) but the technique is scarcely suitable for routine clinical purposes Another serious complication lies in the fact that whilst the amount of freely extractable Porter Silber reacting steroid is small there is in normal urine a large quantity twenty or more times as great of metabolites bound to glucuronide which are not extractable by chloroform or the usual solvents The fraction of this large reserve which spontaneously hydrolyses and becomes chloroform soluble is very variable and is probably dependent on bacterial contamination in the urine sample

These complications account for much greater difficulty with urine extracts than with plasma and as a result it is doubtful whether urinary free steroid estimations are of appreciable clinical value A critical survey of the difficulties of applying the Porter Silber method to urine was made by Vestergaard (1953)

In an attempt to overcome these objections Reddy Jenkins and Thorn (1952) used the fact that butanol is a good extracting solvent for the steroid glucuronides The original method had many theoretical and practical objections and as much as half the chromogenic steroid being measured might be lost in the washings Even with subsequent modifications there are still many objections especially the large quantities of non specific interfering chromogens which Reddy (1954) admits result in high blank values compared with which the colour due to corti costeroid may be only a small percentage of the total

The unreliability of the method in practice has been reported by several workers Smith Mellinger and Patti (1954) found zero or even negative values in apparently normal subjects Marks and Leftin (1954) reported that potassium iodide and paraldehyde could both give false positive readings Similarly Lampe Hintzen and Veld (1955) found a strongly interfering chromogen in the urine of patients taking sylphamerazine

Lampe Hintzen and Veld (1956) have compared the results of the method with the ketogenic steroid method of Norymberski Only a very rough correlation exists between the two It would seem that the Reddy method is more sensitive in revealing adrenal insufficiency though in revealing over action *as in Cushing s syndrome there is little difference between the two*

THE DIAGNOSTIC USE OF ADRENAL INHIBITION

When cortisone is administered to a person with intact adrenal glands it causes a pronounced inhibition of cortical activity probably by the inhibition of corti cotrophin production This can be used as a clinical test as to whether a given gland is under endocrine control The ability of cortisone to inhibit adrenal function was first put to therapeutic use by Wilkins *et al* (1952) who showed that with this hormone the hyperplasia of pseudo hermaphroditism could be brought under proper control

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paper chromatograms The method has been studied critically by Nowaczynski *et al* (1956)

It is desirable because of the technical difficulties involved that any urine sample for assay should be sent by prior arrangement to a centre where such assays are regularly carried out as assays by either method are likely to lead to erroneous results if performed by the inexperienced

Whilst high outputs of aldosterone have been reported in several proved cases of primary aldosteronism (Cope and Llaurado 1954 Milne Muehrcke and Aird 1957) in others urine assay gave a normal result In the diagnosis of primary aldosteronism therefore reliance must be placed mainly on the clinical condition and the biochemical investigation of the potassium losing state (Aird Milne and Muehrcke 1956 Cope and Milne 1945)

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symptoms are controlled more rapidly by the hormones than by salicylates but patients tend to relapse when the drugs are withdrawn In an attempt to solve the problem a co ordinated study was undertaken at 12 British Canadian and American centres The results showed that there was no long term advantage of cortisone or corticotrophin over salicylates These results have been criticized on the grounds that the hormone dosage was too low and a number of American observers have expressed the opinion that the hormones in a relatively high dosage for 3 months or longer are more likely to reduce the incidence of cardiac damage than are salicylates On the other hand British physicians who deal with rheu matic fever feel that hormones have no advantage over salicylates

COMPLICATIONS OF LONG TERM STEROID THERAPY

In the treatment of rheumatoid arthritis with cortisone or corticotrophin for longer than a few weeks it is almost inevitable that side effects of minor or major nature will occur They may be abolished by lowering the dosage but in some cases it may not be possible to avoid them entirely at a dosage which gives adequate suppression Only by the continual measuring of improvement against side effects can the physician decide whether long term treatment is justified

Peptic ulceration

This is the complication most feared by physicians who have used long term cortisone and corticotrophin treatment The exact mechanism by which ulceration is produced is not yet known However numerous studies by Gray *et al* (1951) have established the clinical importance of the effects of adrenal steroids on gastric function Cortisone has been shown both in normal persons and in those with Addison s disease to cause an increase in basal and nocturnal hydrochloric acid secretion and this is associated with a pronounced rise in the pepsin content of gastric juice The latter is reflected in a greatly increased urinary excretion of uropepsin the output of which is frequently raised to the high levels encountered in peptic ulceration

The administration of cortisone to patients who have undergone vagotomy has been found to produce an increase in uropepsin excretion It thus appears that the action of cortisone on the cells of the gastric mucosa may be a direct one and not mediated through nervous pathways A high correlation exists between the urinary content of uropepsin and 17 hydroxycorticosteroids after adrenocortical stimulation

Masking of symptoms

The practical risk with patients who develop peptic ulceration on cortisone or corticotrophin is that the digestive symptoms may be masked and the first indication may be haemorrhage or perforation The result is that physicians with experience of long term treatment with steroids have come to place importance on minor gastric symptoms and always to investigate them at an early stage

It has been advocated in many text books that if peptic ulceration occurs steroids should be withdrawn In practice this step may be too drastic as it means that patients who have regained their independence will be condemned to renewed crippledom By investigating and treating symptoms of dyspepsia at an early

CHAPTER 14

CORTISONE AND CORTICOTROPHIN IN THE TREATMENT OF RHEUMATIC DISORDERS

OSWALD SAVAGE

THE FIELD FOR STEROID THERAPY IN RHEUMATIC DISORDERS

Rheumatoid arthritis

THERE is a tendency for some cases of rheumatoid arthritis to be mild while in others there are long periods of remission Because of the risk of complications with steroid therapy the likelihood of prolonged or indefinite treatment and the necessity for constant supervision it is considered in Great Britain unwise to undertake steroid treatment until other methods have been tried As the Medical Research Council–Nuffield Committee (1954) and the Empire Rheumatism Council trials have shown many cases improve with a regime of salicylates and in some cases a period of rest alone will be followed by a remission On the other hand if steroid therapy is to be used it should be started before there is permanent damage and deformity as it will have no effect on cartilage erosion or on a joint capsule contracted by fibrosis There are certain absolute contra indications to the use of long term steroid therapy active or recent tuberculosis diabetes mellitus severe psychological upsets or psychosis peptic ulceration and severe joint damage

In certain cases where correction of joint deformity such as flexion deformity of the knee is to be undertaken by means of a manipulation or serial splinting or extensive rehabilitation it may be helpful to give cortisone or corticotrophin to suppress inflammation during this period Sometimes it is hard to decide whether steroid therapy should be used because of the difficulty in estimating the degree of improvement which might be obtained In such circumstances treatment with cortisone or corticotrophin can be given for a few weeks and then withdrawn slowly if there is not sufficient improvement to justify long term therapy

Ankylosing spondylitis

There is a small place in this condition for the use of steroids during exacerbation of acute back pain For routine treatment deep x ray therapy at any rate for one course is considered to have more effect on the disease process and for spinal pain phenylbutazone has proved more effective than cortisone

Rheumatic fever

The place of cortisone and corticotrophin in treatment has not yet been decided The essence of the problem is whether hormones are more effective in prevent ing cardiac damage than are salicylates There is no doubt that fever and joint

Surgeons have been impressed by the fact that in rabbits wound healing is inhibited by very large doses of steroids and they may be reluctant to increase dosage for fear of wounds not healing normally However the doses of steroids used in the wound healing experiments were excessive and bear no relation to present human dosage In practice fatalities have been known to result from adrenal failure when cortisone has been withdrawn before even minor surgical procedures

It should be routine practice to give extra cortisone to cover surgery or other emergencies and it is recommended that 100 milligrams be given intramuscularly 24 hours before and on the day of operation more should be given before major operations such as adrenalectomy and its withdrawal should be slower The objective should be to provide a depot of adrenal steroid in case of body need particularly if oral administration may not be possible over the operation period Intravenous hydrocortisone should also be available for use in case of a dangerous fall of blood pressure after operation

Obesity

This may be a troublesome problem in long term treatment particularly in women at the menopause Both cortisone and corticotrophin cause an increase in appetite and patients may have difficulty in restraining themselves from over eating Most patients with rheumatoid arthritis are underweight and it has been found by experience that once the weight has returned to normal and there is a tendency to gain more it is wise to impose dietary restrictions particularly with regard to carbohydrate Many patients with rheumatoid arthritis have some damage to the knee and foot joints and obesity adds a factor of mechanical strain to their lower limbs which may well increase their symptoms

Osteoporosis

Albright (1942) has shown that the anti anabolic protein deficiency effects of oxysteroids can result in osteoporosis and this can be serious in a disease such as rheumatoid arthritis which may be accompanied by osteoporosis Long term steroid therapy therefore should not be undertaken in cases where it is severe for fractures of vertebrae and long bones have been known to occur It is advisable in such cases to give a high protein diet and androgen therapy can also be employed It should be remembered too that patients who have been confined to chair or bed with rheumatoid arthritis and who are on cortisone treatment must be rehabilitated with care if osteoporosis is present

Sodium retention

In animals small doses of cortisone or corticotrophin cause sodium retention by resorption by the renal tubules and also diminution of both chloride and sodium excretion in sweat and saliva With such treatment therefore a careful watch should be kept for the onset of oedema which tends to occur first in feet and ankles It is important to recognize this early particularly where cardiac function is poor as it may progress to congestive oedema unless corrected With large doses of these drugs sodium retention with oedema can occur within a few days This may be corrected by a spontaneous diuresis after a week or two but it can persist and

stage so that ulceration is demonstrated before erosion is advanced it has been possible to produce healing by medical treatment in a number of cases and without withdrawing steroids Occasionally where the ulcer has failed to heal completely under medical measures partial gastrectomy has been undertaken and the steroids continued with success

Gastric haemorrhage

Patients on cortisone or corticotrophin who become anaemic should be investigated for the possibility of intestinal bleeding If haemorrhage from a peptic ulcer occurs there is a tendency for this to continue even to a fatal issue possibly because of the alteration in blood coagulation caused by steroids Because of the possibility of a continued gastric haemorrhage it may be wiser to have an area of ulceration removed early otherwise a surgeon may be faced with an emergency operation on an exsanguinated patient Such a complication as gastric haemorrhage from a peptic ulcer is not common in the treatment of rheumatoid arthritis with cortisone or corticotrophin but it is one which the physician has to regard as a dangerous possibility It is therefore important to demonstrate it and treat it at an early stage

Suppression of inflammation and the masking of infections

The original observations of Hench on the dramatic effect of cortisone and corticotrophin in rheumatoid arthritis called attention to the capacity of adreno cortical hormones to suppress inflammation This suppression was manifested clinically in joints by a rapid lessening of the classical signs and histologically by a decreased number of plasma cells and lymphocytes a reduction of papillary tufting a decreased deposition of fibrin and diminution of necrosis and oedema in the synovial membrane

Cortisone and corticotrophin have been shown in numerous studies to be capable of suppressing the inflammatory response to a wide variety of inciting agents including chemical irritants foreign proteins and micro organisms These hormones also inhibit the general systemic signs of inflammation such as fever and toxaemia Much work has been done in investigating the effect on antibody formation but there are no conclusive studies showing that this is altered

Signs of inflammation are suppressed so that though patients will feel ill from for instance a pulmonary or renal infection the fever and clinical signs are reduced So far as is known an antibiotic or sulphonamide preparation can be used as effectively as in the normal way and without alteration in steroid dosage

Adrenal cortical suppression and failure in emergency

The capacity of the body to respond to stress is profoundly modified by deficiency or excess of adrenocortical hormones Vulnerability to stress is a characteristic of Addison s disease and it can be combated at ordinary levels by relatively small quantities of oxysteroids such as cortisone During long term cortisone treatment involutionary changes tend to occur in the adrenal cortex and its ability to react to stress is diminished This may also occur to some extent with corticotrophin treatment when the cortex cannot respond to further stimulation This means that in such cases an increased dose of cortisone should be given in the event of inflammation or operation

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be a serious and definite danger With the smaller doses of these steroids now employed in the long term treatment of rheumatoid arthritis severe oedema is uncommon When it occurs it can be corrected by the use of mercurial diuretics It is often the practice to eliminate salt from the diet in an attempt to avoid this complication

Potassium depletion

Many of the adrenal cortical steroids cause increased excretion of potassium and this may be a complication of long term treatment The symptoms of such hypokalaemia and alkalosis may be obscure Generalized weakness accompanied by diffuse abdominal pain may lead to a suspicion of this complication which can be confirmed by finding the typical flattening of the T wave in the electrocardio gram and a low potassium level in the blood The condition can be corrected by giving potassium chloride by mouth and lowering the steroid dosage

Glycosuria

With prolonged administration of cortisone diminution in glucose tolerance and consequent glycosuria may be produced As pancreatic diabetes may be worsened by adrenal steroid administration it is important to exclude diabetes mellitus before steroid therapy is undertaken

Psychological disturbances

Definite improvement in the mental outlook of patients with rheumatic disorders almost always occurs while cortisone and corticotrophin are being administered Sometimes an exaggerated sense of well being may develop It is natural in a chronic disease such as rheumatoid arthritis with the fear of permanent crippling always present for dramatic relief of pain and disability to result in a return of self confidence An optimistic elated mood amounting to euphoria often *develops with an acceleration of the tempo of mental and physical activity* It is important that a careful evaluation of the patient s personality and family history should be carried out when hormone treatment is contemplated If there is any question of psychosis it should not be prescribed

PATTERN OF RESPONSE TO STEROID THERAPY IN RHEUMATOID ARTHRITIS

During the early trials of cortisone and corticotrophin large doses were given and the response was dramatic Patients stated that they felt as though their limbs were unlocked and those who were bedridden or confined to a chair were able to move easily in a few days The pain and inflammation were suppressed *rapidly and they* could feed and dress themselves within 48 hours and this after being dependent on other people for months or years (Fig 4) It was soon clear however that side effects also occurred rapidly and that prolonged treatment with large doses was impracticable In addition rapid transition from crippling to mobility resulted in weak muscles being over used and over strained and bones which had become osteoporotic from the disease were liable to fracture under sudden stress It has therefore become the practice to start treatment with smaller doses and so improvement is more gradual and side effects avoided

Most physicians using steroids in rheumatoid arthritis now give an initial dose of 50 milligrams of cortisone or 20 units of corticotrophin The dose of cortisone may be raised to 75 milligrams a day if there is no response and to 100 milligrams a day for short periods but in most cases this dosage will produce side effects if administered for long periods

Corticotrophin has the disadvantage of having to be injected On the other hand by measuring the output of urinary 17 hydroxycorticosteroids the level of adrenal stimulation can be estimated and the dosage so regulated Patients have

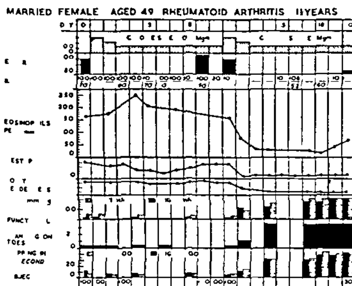


FIG 4—Showing the continued improvement throughout a trial with cortisone (*By courtesy of the Editor of the British Medical Journal*)

injected themselves subcutaneously each day with satisfactory results for as long as 4 years

With moderate dosage of steroids the pattern of improvement is slower but satisfactory It is usually a week or more before improvement can be measured though the lassitude accompanying the rheumatoid arthritis may disappear more quickly Joint tenderness lessens but is not usually abolished joint swelling is diminished but it too may not entirely disappear and movement increases though the result is dependent on the amount of permanent joint damage present In a satisfactory response the power of the grip usually doubles and in general the ability to perform simple movements such as dressing and light work is restored Function improves more than objective signs and patients should regain their independence and be able to return to work though the disease is still active and only suppressed Organized rehabilitation with exercises is necessary in the early stages of treatment

In the early days of these drugs the fear was expressed that dose requirements would increase but this has not proved to be the case It is the accepted practice

be a serious and definite danger With the smaller doses of these steroids now employed in the long term treatment of rheumatoid arthritis severe oedema is uncommon When it occurs it can be corrected by the use of mercurial diuretics It is often the practice to eliminate salt from the diet in an attempt to avoid this complication

Potassium depletion

Many of the adrenal cortical steroids cause increased excretion of potassium and this may be a complication of long term treatment The symptoms of such hypokalaemia and alkalosis may be obscure Generalized weakness accompanied by diffuse abdominal pain may lead to a suspicion of this complication which can be confirmed by finding the typical flattening of the T wave in the electrocardiogram and a low potassium level in the blood The condition can be corrected by *giving potassium chloride by mouth and lowering the steroid dosage*

Glycosuria

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Psychological disturbances

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Intra articular injections

Intra articular injections have become routine treatment in rheumatoid arthritis and in some cases of osteoarthritis where there is an inflammatory effusion The usual precautions against sepsis must of course be taken when a joint is injected There are two drawbacks to this type of treatment

Technical difficulties

The first is the difficulty and pain of injecting certain small joints such as those of the fingers and feet French rheumatologists are enthusiastic about hydro cortisone injection of Heberden s nodes in the terminal phalangeal joints but it can be a most painful procedure Larger joints such as the knee ankle and shoulder are relatively easy to inject particularly where there is an excess of joint fluid The knee is the joint most commonly treated by this method The hip joint is extremely difficult to inject but recently a simplified method of approach has been described by Landsmeer and Koumans (1954) A new method of intra articular injection by a powerful hypospray which eliminates the use of a needle has been described by Ziff Contreras and Schmid (1956)

Short action of hydrocortisone acetate

The second drawback arises from the fact that the effect of local hydrocortisone acetate only lasts a short time probably a few days so that the procedure may have to be repeated at frequent intervals if there is recurrence of inflammation It is not feasible to inject even large joints such as the knee more than once a month and so this type of treatment can only be repeated on a limited number of occasions Other substances have been tried in the hope of finding one which would have a longer lasting anti inflammatory action but without success

In spite of the short action of hydrocortisone and the practical difficulty of injecting certain joints this treatment is valuable for soft tissue lesions for its intra articular effect mostly in rheumatoid arthritis and occasionally for other inflammatory joint conditions No advantage has been found in employing more than 50 milligrams (2 millilitres) for a single joint and in small joints or localized lesions less may be used Care must be taken that it is never employed where there is a possibility of the disease being tuberculous

THE CORTISONES

Pharmacological research

Chemical research has aimed at providing other steroids with an anti inflam matory and antirheumatic effect without the side effects and the following sub-stances have been tried in rheumatoid arthritis

Hydrocortisone acetate

This is considered to be the main anti inflammatory adrenal glucocorticoid and as its local effect is more pronounced than that of cortisone it has become the steroid of choice for intra articular and soft tissue injections Boland (1955) investigated the antirheumatic effects of hydrocortisone given orally and found that the pattern of improvement was the same as with cortisone but that its potency was 50 per

that adequate suppression of symptoms and signs should be obtained with the lowest possible dosage *Periodic attempts to reduce the dose should be made in a* long term programme of treatment

Both cortisone and corticotrophin correct the anaemia of rheumatoid arthritis The sedimentation rate usually falls initially but tends to rise again and levels off above normal but below the original figure Rose s differential agglutination reaction is not restored to normal

ROUTINE SUPERVISION

The routine management of cases on long term steroid therapy is best carried out at a clinic For patients on small doses in whom it may be difficult to be sure whether the anti inflammatory effect is satisfactory methods of measurement such as strength of grip amount of joint tenderness and the carrying out of certain movements which can be timed are useful

Regular weighing testing for glycosuria and for oedema *and estimation of the* blood pressure should be carried out and patients should be questioned about dyspepsia Analgesics are allowed and it is helpful to know the dose being used daily Estimation of the sedimentation rate is a useful guide as it tends to return to normal if the underlying disease is going into remission and tends to rise if the arthritis is becoming more active in which case a higher dose of steroid is necessary It is probably wise to have a chest x ray taken every 6 months to exclude any tendency to reactivation of an old tuberculous focus In the early stages while the dose is being adjusted to a working minimum patients should attend weekly *Once they are stabilized it has been found sufficient to see them every 4 or 6 weeks*

LOCAL HYDROCORTISONE

Hydrocortisone acetate appears to have the most marked anti inflammatory action *of all the oxysteroids when used locally Hollander et al (1951)* have shown that there is a substantial reduction of leucocytes and especially of polymorphonuclear leucocytes in an inflammatory joint effusion within a few hours of the intra articular injection of hydrocortisone

It has also been shown that there is a change in the mucin fraction of the synovial fluid with a rise in viscosity It would appear that the effectiveness of an intra articular agent is related to its solubility in the fluid Hydrocortisone acetate is the least soluble steroid and the most effective Studies on the recovery of the steroid from the joint fluid after injection indicate that it is taken up by the synovial cells It has been shown that after a few minutes only a small amount of hydro cortisone can be recovered from the joint fluid demonstrating its rapid absorption by these cells Other steroids have a similar action in joint fluid but are much less effective than hydrocortisone acetate

This local anti inflammatory effect has been widely used in the treatment of a number of *conditions associated with the rheumatic disorders Local inflammatory* conditions of the soft tissues such as epicondylitis (tennis elbow) subacromial bursitis (periarthritis of the shoulder) inflammation of the tendo achillis and stenosing tenosynovitis (de Quervain s disease) have responded to accurately placed injections of hydrocortisone acetate so saving weeks or months of physiotherapy

amount of 17 hydroxycorticosteroid in urine This has been a great step forward as it is now possible to follow the level of adrenal stimulation produced by corticotrophin Using this measurement West and Newns (1953) have shown the possibilities of treating rheumatoid arthritis by continuous adrenal stimulation West (1956) has also pointed out the satisfactory results obtained from using low potency corticotrophin as manufactured at present higher potency preparations will allow more accurate control

Variations in blood hydrocortisone in rheumatoid arthritis

As a result of measurements of the amount of hydrocortisone in the blood which is now possible by the method of Porter and Silber some interesting progress in the knowledge of cortisone metabolism has been made It has been found that there is a diurnal variation in the level of blood hydrocortisone in normal subjects The mean value is high at 8 a m though in some people this is delayed till 10 a m and falls during the next 4 hours After a short rise it falls again and is low between 4 p m and 6 p m Studies throughout the night have shown a low level at 3 a m followed by a rise to the morning peak Investigations on rheumatoid patients have shown that the blood hydrocortisone is high but that there are greater fluctuations throughout the day than in normal subjects

Scientific control of treatment

Estimations of blood hydrocortisone should soon enable the dosage of cortisone to be controlled by scientific calculation instead of by balancing clinical improvement and side effects In the case of corticotrophin estimation of the level of adrenocortical activity should make it possible to avoid over stimulation and to adjust the dose to that required for the suppression of symptoms

In fact as knowledge grows the physician should be able to use the cortisones and corticotrophin more scientifically Nevertheless it must be remembered that since these steroids were introduced in 1948 a large number of patients with rheumatoid arthritis and allied conditions of a type which up to that time had failed to respond to other treatments have been greatly benefited and have been able to get about again and to return to work

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cent greater After further study he concluded that although hydrocortisone was a valuable agent in the management of selected cases of rheumatoid arthritis it was far from an ideal suppressive agent Like cortisone it had many shortcomings chief among which were hormonal side effects especially when dosage requirements were large and the tendency to aggravate certain co existing pathological conditions The development of unresponsiveness in some patients after prolonged administration and the failure to prevent disease progression in an appreciable percentage of cases were also noted Hydrocortisone by mouth in dosages of 70 milligrams or less a day has been found valuable in the long term treatment of cases which tend to develop obesity on cortisone

9 α Fluorohydrocortisone

This steroid has been tested in rheumatic disorders Its anti inflammatory action at least ten times stronger than that of cortisone is accompanied by marked sodium retaining effects and in preliminary studies almost all patients developed signs of fluid retention at an early stage This precluded its use in rheumatoid arthritis

Prednisone and prednisolone

A number of other steroid compounds have been given pilot trials in rheumatoid arthritis but none was satisfactory until the advent of prednisone and prednisolone Most observers agree that these substances are four or five times more potent than cortisone acetate and that the sodium retaining effects are less but it is thought that they have a particular tendency to produce dyspepsia and peptic ulceration It would seem that they are superior to cortisone in the long term management of rheumatoid arthritis and are useful when sodium retaining effects have become troublesome but inferior when there is a tendency to dyspepsia

Corticotrophin

When corticotrophin was first used for the treatment of rheumatoid arthritis there were many disadvantages some of which have now been overcome The early preparations were short acting and had to be given by intramuscular injection several times a day The present long acting preparations usually provide adrenal stimulation for 24 hours or more and can be given subcutaneously Patients under this treatment can inject themselves in the same way as diabetics

SOME ADVANCES AND THE FUTURE

Tests of adrenal activity in rheumatoid arthritis

It has been considered that in the many conditions where cortisone is effective there may be some abnormality of the adrenal cortex but in rheumatoid arthritis all efforts have failed to demonstrate any evidence of adrenal failure Although patients with this condition frequently have a low level of adrenal cortical activity the test measurements are within the normal range but the present methods of measuring adrenal activity are not satisfactory and in the main consist of estimating adrenal metabolites in the urine In fact the estimation of 17 ketosteroid output which includes androgen and oestrogen end products was the only method available until Norymberski introduced his practical method of estimating the

amount of 17 hydroxycorticosteroid in urine This has been a great step forward as it is now possible to follow the level of adrenal stimulation produced by corticotrophin Using this measurement West and Newns (1953) have shown the possibilities of treating rheumatoid arthritis by continuous adrenal stimulation West (1956) has also pointed out the satisfactory results obtained from using low potency corticotrophin as manufactured at present higher potency preparations will allow more accurate control

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CHAPTER 15

CORTICOTROPHIN AND CORTISONE IN THE SUPPRESSION OF ALLERGIC AND PARARHEUMATIC DISORDERS

F DUDLEY HART

INTRODUCTION

THE ALLERGIC disorders common in Great Britain are in the main more of a major nuisance to the patient than serious diseases dangerous to life Asthma is an exception though only relatively rarely does it prove fatal but hay fever urticaria and the like are productive of many annoying and distressing symptoms which are eventually self terminating if left untreated Provided that no contra indications exist treatment with corticotrophin or cortisone or one of its analogues hereafter termed steroid therapy may make life incomparably more comfortable until the natural remission of symptoms occurs Short term treatment of this kind may be most helpful in selected patients and in the usual dosage

The term pararheumatic is here used to indicate those disorders which bear some resemblance to rheumatoid arthritis but which are essentially very different clinical and therapeutic problems—disseminated lupus erythematosus polyarteritis nodosa scleroderma and dermatomyositis The interesting and allied disorder cranial arteritis has also been included except for this disease the group is characterized by progressive crippling and a very real risk to life The use of steroid therapy in such conditions as disseminated lupus erythematosus is usually essential in all but mild cases for quite apart from relief of symptoms a progressive downhill course is often seen if it is withheld Whatever therapy is given the prognosis remains bad in the pararheumatic disorders steroid therapy for instance does not prevent the development of the renal changes which so often close the scene

MODE OF ACTION OF ADRENAL STEROIDS

Robson and Keele (1956) have summarized the ways in which steroid therapy may be effective though they note that no really satisfactory explanation has yet been given as to its action Their data may be grouped under three main headings

(1) Effects on enzymatic and metabolic processes
(2) (a) Effects on mesenchymal tissues
 (b) Effects on antigen antibody reactions and the process of immuniza tion
(3) General theories attempting to explain all the observations within one main idea

The enzymatic and metabolic effects have been reviewed by Lieberman and Teich (1953) Although a la.ge amount of information has accumulated we are still far from

a clear conception of what goes on in the different enzyme systems under the influence of the various steroid hormones The fact that steroid therapy reduces the signs of inflammation has been well demonstrated both in man and the experimental animal but how it does so is still unknown Changes in vascular tone and capillary permeability under treatment have been demonstrated by a number of workers (Ebert and Wissler 1951 Cook and MacDonald 1951) and Swingle and Parkins (1935) noted the relation ship of adrenal hormones to capillary permeability in infusion experiments in adrena lectomized dogs Turning to immunity and allergy Rich *et al* (1950) produced cardiovascular lesions in rabbits by protracted anaphylactic reactions these changes could be inhibited by cortisone and corticotrophin Ebert and Wissler (1951) using the rabbit ear chamber technique found that cortisone produced a marked reduction in the degree of vascular damage caused by serum sickness Vascular tone was better maintained the endothelium retained its normal appearance and swelling was suppressed Duke Elder and Ashton (1951) reviewing the literature and more recent work on this subject in the eye noted that while no effect on the permeability of normal capillaries had been observed the increased permeability characteristic of inflammation was significantly lessened by cortisone and the exudative phenomena inhibited in conditions unassociated with allergic states It seems clear on the existing evidence that it is inflammation and not the allergic state which is essentially affected by steroid therapy

Robson and Keele (1956) pointed out that cortisone does not act as an antihistamine in that it does not produce its action by preventing the effect of one of the end products of the antigen antibody reaction Corticotrophin does not influence the development of bronchospasm induced by histamine in guinea pigs (Friedlaender and Fried laender 1950) nor according to Herschfus *et al* (1950) in asthmatic patients

There is considerable evidence that steroid therapy does not inhibit anaphylaxis in the few experiments in which acute anaphylaxis was successfully prevented (Nelson *et al* 1950 Hoene 1952) large doses of hormone were used and the assumption was that a block in tissue responsiveness was effected rather than an inhibition of antigen antibody union There is also evidence that intense adrenocortical stimulation does not significantly alter the ability of tissues to release histamine like substances nor does it prevent them responding either to these substances or to histamine itself (Grob *et al* 1952) It seems unlikely therefore that the effectiveness of steroid therapy in allergic states is related to any effect on these particular mechanisms

Effect upon local allergic phenomena

The effect of cortisone and corticotrophin upon local allergic phenomena has also been intensively investigated In the past the tuberculin reaction has been inhibited by comparatively large doses of these hormones and it seems that the Schwartzman phenomenon may also be blocked These effects appear to depend essentially on interference with some phase of tissue reactivity Recently interesting work in this respect has been reported by Citron and Scadding (1957) They injected cortisone acetate locally with the tuberculin used for the intradermal test and found that in active pulmonary tuberculosis and in subjects of high tuberculin sensitivity cortisone greatly inhibited the reaction but the inhibitory effect lessened progressively with diminishing tuberculin sensitivity until in subjects of low tuberculin sensitivity there was no inhibitory effect at all In healthy tuberculin insensitive subjects cortisone and tuberculin caused no reaction but in tuberculous subjects whose skin had been rendered insensitive to tuberculin by desensitization with tuberculin cortisone and tuberculin produced reactions in 70 per cent Similarly in patients with sarcoidosis insensitive to tuberculin cortisone and tuberculin produced reactions in 50 per cent They suggested that cortisone has two specific effects upon the tuberculin reaction an

anti inflammatory effect which tends to diminish the reaction and a tuberculin retaining effect which tends to enhance it In subjects of very low tuberculin sensitivity the anti inflammatory effect is subordinate to the tuberculin retaining effect and this makes possible the detection of a degree of tuberculin sensitivity too low to be detected by tuberculin alone

There is evidence that antibody synthesis may be depressed by corticotrophin or cortisone but the depression is slight and occurs only when very large amounts of hormone are given The clinical efficacy of cortisone probably bears little relation to antibody inhibition except in certain special disorders such as acquired haemolytic anaemia

In summary it seems that corticotrophin and cortisone produce their ameliorating effects in allergic disorders by inhibition of the inflammatory tissue response rather than by any effect on antigen or antibody or both The therapeutic response is similar therefore to that in the treatment of inflammatory disorders not based on hypersensitivity As Thorn *et al* (1953) stated In all likelihood the common factor in the multiple actions of cortisone and corticotrophin on the bodily mechanisms of defence is a modification of the reactivity of mesenchymal tissue

CHOICE OF PREPARATION

The relative merit of different preparations is partly a matter of individual experience Certain allergic conditions may be accompanied by a tendency to bruise Should there be a platelet or some other defect in the clotting process intramuscular therapy may cause painful haematomas so that in such cases oral preparations are preferable Corticotrophin occasionally produces allergic tissue reactions and rarely a more general allergic reaction Some patients respond more satisfactorily to one substance than to another and the clinician must decide in the individual case which drug to prescribe For short term therapy cortico trophin is useful and may often be preferred as it usually produces quick and effec tive relief from distressing symptoms There is now considerably less variation in the efficiency of different batches of corticotrophin though variation still occurs With cortisone and its analogues variation is not found Treatment however for more than a few days may produce temporary adrenal hypoplasia though this has perhaps been somewhat over emphasized in the past

DOSAGE

In the acute self terminating episode of an allergic disorder so severe as to carry a risk to life the dose to use is the dose which works Higher dosage than that usually given is permissible if in all probability therapy will be reduced *drastically* within a few days up to 100 milligrams (or even more) a day of cortico trophin given intramuscularly in divided dosage or 200–300 milligrams of corti sone or 40–60 milligrams of prednisone or prednisolone However even over short periods of 2–3 days such high dosage may produce serious side effects such as gastric haemorrhage or perforation Only rarely is such high dosage necessary and then only in the face of a very severe disorder carrying a real risk to life dosage may usually be reduced after 24–48 hours of such intensive therapy Some conditions may completely fail to respond to the usual dosage levels in which case a high initial dosage as above becomes necessary Unfortunately at safe

dosage levels—100 *milligrams or less of cortisone, 80 milligrams or less of hydro* cortisone 20 milligrams or less of prednisone or prednisolone or 40 milligrams or less of corticotrophin a day—the therapeutic effect is often inadequate

In grave emergency hydrocortisone (free alcohol) or corticotrophin may be given by intravenous infusion though this is rarely necessary for the action of cortisone hydrocortisone prednisone or prednisolone by mouth is rapid enough for the ordinary case Corticotrophin may be given intramuscularly every 1–4 hours for the first few injections this method of attack brings most acute allergic conditions under control fairly rapidly and dosage may then be continued once or twice a day using the long acting gel

STEROID THERAPY IN MINOR ALLERGIC DISORDERS

Corticotrophin and cortisone or one of its analogues are effective and useful substances in the treatment of minor allergic reactions but it may well be asked if *it is really necessary to use them for such conditions The answer is in the negative* if very high doses are required but perhaps the reverse if unpleasant symptoms can be relieved by small doses and without side effects

Hay fever

It has been reported that cortisone combined with hyposensitization produces greater relief in patients with hay fever than hyposensitization alone Schwartz *et al* (1952) gave 100 milligrams of cortisone in divided doses daily during the height of the hay fever season with success most clinicians today would consider this dosage excessive if continued for more than a week or two Recently Friedlaender (1956) submitted the report on new drugs of the Committee of the Research Council of the American Academy of Allergy Over the period 1955–1956 110 patients with hay fever were treated with prednisone and 111 with prednisolone With prednisone results were excellent in 55 (50 per cent) good in 35 (31 8 per cent) fair in 5 (4 5 per cent) and poor in 15 (13 65 per cent) Similar results were obtained with prednisolone 16 patients received both drugs interchangeably the results being equal in 14 Good as these results are it is to be noted that the daily dose needed to suppress symptoms in responsive subjects was between 5 and 140 milligrams It averaged 45 8 milligrams daily for prednisone and 41 1 milligrams for prednisolone The time necessary to effect suppression was from 4 to 168 hours averaging 40 2 hours for prednisone and 33 5 hours for prednisolone Minimum daily doses to maintain suppression varied from 2 5 to 40 milligrams Side effects occurred in 10 9 per cent of patients receiving prednisone and in 5 4 per cent receiving prednisolone they included mental stimula tion gastro intestinal distress muscular pains headache palpitations tachycardia urinary frequency polyuria and chest pain Therapy had to be discontinued in 7 patients Because of side effects some severe (for example reactivation of peptic ulcer and gastro intestinal bleeding) the Committee stated It is felt therefore that treat ment of hay fever with these newer as well as older steroids should be limited only *to those severe cases that are unresponsive to desensitization or fail to achieve relief* from the more conservative forms of symptomatic therapy This seems sound advice

The general trend now is to reserve steroid therapy for incapacitated patients unresponsive to simpler measures *They should be free of any of the accepted* contra indications and then should only be treated in safe dosage after perhaps 1–2 days of a higher initial dosage

ALLERGIC SKIN CONDITIONS

Angioneurotic oedema atopic dermatitis and some cases of drug allergy may show striking response to steroid therapy but it must be understood that the cause should always be found first and if possible eliminated conventional methods should not be overlooked Steroid therapy has been successful only 3 or 4 days therapy being usually necessary in the pruritic and inflammatory stages of allergic contact dermatitis as seen from hair dyes primulas and local antiseptics and also in poison ivy dermatitis where other methods have failed

Angioneurotic oedema (giant urticaria)

Symptoms may be most unpleasant and frightening in this condition particularly if swelling of the tongue takes place and though it usually subsides within 2–14 days control during this period is important

Pillsburg and Urbach (1954) giving a long list of skin disorders with their reactions to steroid therapy noted that results in angioneurotic oedema were satisfactory and that it was justifiable to use such therapy in severe cases particularly with the risk of laryngeal *involvement* Mitchell Heggs (1956) has observed that *allergic skin reactions may* respond to cortisone although intractable to antihistamines and that cortisone may render such conditions amenable to treatment with antihistamines Beiglbock and Brummond (1956) using an initial dose of 30 milligrams of prednisone followed by a gradual reduction in dosage obtained good results in all of 7 patients with urticaria Rein and Bodian (1956) obtained similar results with a slightly higher dosage

It would seem therefore that in the opinion of most workers this unpleasant condition can be effectively controlled in the majority of cases If laryngeal involvement occurs high dosage is indicated possibly by the intravenous route until the acute symptoms come under control when dosage can be reduced In all cases dosage should be reduced stepwise and withdrawn gradually when it is considered that the allergic condition has subsided With rapid withdrawal if natural remission has not occurred symptoms may reappear rapidly and sometimes alarmingly In some cases urticaria may persist for a considerable time and if such cases cannot be controlled after one or two weeks except by large amounts of steroid therapy dosage must be reduced gradually to safe levels and withdrawn In this condition too it must be emphasized that search must be made for the allergen often a food or drug and this must be eliminated if found before steroid therapy is withdrawn

Chronic urticaria

This condition is in the opinion of Pillsburg and Urbach much less amenable to cortisone therapy their results were so poor as to make such treatment hardly *worth while They agree with Sulzberger et al (1951)* that these urticarial lesions are not suppressed by cortisone to any significant extent in most patients although on discontinuing therapy the condition often worsened Other forms of steroid therapy may be tried in resistant cases and occasionally success has followed treatment with corticotrophin when cortisone has failed

Atopic dermatitis

Although this condition occurs in persons of allergic stock and starts commonly

dosage levels—100 milligrams or less of cortisone 80 milligrams or less of hydro cortisone 20 milligrams or less of prednisone or prednisolone or 40 milligrams or less of corticotrophin a day—the therapeutic effect is often inadequate

In grave emergency hydrocortisone (free alcohol) or corticotrophin may be given by intravenous infusion though this is rarely necessary for the action of cortisone hydrocortisone prednisone or prednisolone by mouth is rapid enough for the ordinary case Corticotrophin may be given intramuscularly every 1-4 hours *for the first few injections this method of attack brings most acute allergic* conditions under control fairly rapidly and dosage may then be continued once or twice a day using the long acting gel

STEROID THERAPY IN MINOR ALLERGIC DISORDERS

Corticotrophin and cortisone or one of its analogues are effective and useful substances in the treatment of minor allergic reactions but it may well be asked if it is really necessary to use them for such conditions The answer is in the negative if very high doses are required but perhaps the reverse if unpleasant symptoms can be relieved by small doses and without side effects

Hay fever

It has been reported that cortisone combined with hyposensitization produces greater relief in patients with hay fever than hyposensitization alone Schwartz *et al* (1952) gave 100 milligrams of cortisone in divided doses daily during the height of the hay fever season with success most clinicians today would consider this dosage excessive if continued for more than a week or two Recently Friedlaender (1956) submitted the report on new drugs of the Committee of the Research Council of the American Academy of Allergy Over the period 1955–1956 110 patients with hay fever were treated with prednisone and 111 with prednisolone With prednisone results were excellent in 55 (50 per cent) good in 35 (31 8 per cent) fair in 5 (4 5 per cent) and poor in 15 (13 65 per cent) Similar results were obtained with prednisolone 16 patients received both drugs interchangeably the results being equal in 14 Good as these results are it is to be noted that the daily dose needed to suppress symptoms in responsive subjects was between 5 and 140 milligrams It averaged 45 8 milligrams daily for prednisone and 41 1 milligrams for prednisolone The time necessary to effect suppression was from 4 to 168 hours averaging 40 2 hours for prednisone and 33 5 hours for prednisolone Minimum daily doses to maintain suppression varied from 2 5 to 40 milligrams Side effects occurred in 10 9 per cent of patients receiving prednisone and in 5 4 per cent receiving prednisolone they included mental stimulation gastro intestinal distress muscular pains headache palpitations tachycardia urinary frequency polyuria and chest pain Therapy had to be discontinued in 7 patients Because of side effects some severe (for example reactivation of peptic ulcer *and gastro intestinal bleeding) the Committee stated It is felt therefore that* treatment of hay fever with these newer as well as older steroids should be limited only to those severe cases that are unresponsive to desensitization or fail to achieve relief from the more conservative forms of symptomatic therapy This seems sound advice

The general trend now is to reserve steroid therapy for incapacitated patients unresponsive to simpler measures They should be free of any of the accepted contra indications and then should only be treated in safe dosage after perhaps 1–2 days of a higher initial dosage

but is now seen with many new therapeutic agents It is usually severe and it may occasionally prove fatal After withdrawal of the offending agent therapy must be energetic and the dose of steroid therapy to be given is the dose which proves effective In cases of gold toxicity treated with dimercaprol (BAL) steroid therapy can also be given for not only will it reduce the inflammatory features of the gold intoxication but it may also damp down the occasional side effects of dimercaprol The length of the course of steroid therapy depends on the case and the nature of the causative drug In the Medical Research Council (1954) report steroid therapy was said to control or cure most cases of idiopathic exfoliative dermatitis other than the variety which follows psoriasis

LOCAL THERAPY IN ALLERGIC DISORDERS

Hydrocortisone has been widely used as a locally applied anti inflammatory agent in dermatological disorders and cortisone and hydrocortisone in ocular conditions Examples of true allergic reactions in the eye are allergic conjunctivitis and atropine sensitization and in the skin localized contact dermatitis Extensively used as are drops ointments and lotions and effective as they are in reducing the unpleasant inflammatory features of many conditions in both sites warnings have been repeatedly given as to the dangers of infection in the eye with risk of corneal perforation As regards the skin Mitchell Heggs (1956) reported the effects of topical application of hydrocortisone as very variable and frequently no better than application of simple control ointments In both sites there has in Great Britain in the past year perhaps been an over optimistic use of these substances as local agents the future trend may well be towards a more critical application There is great need for valued and controlled studies of these substances as local agents both in dermatology and ophthalmology Used critically and carefully the local application of hydrocortisone seems to have a real place in both spheres

ASTHMA

It appears to be the effect of steroid therapy on inflammatory tissue rather than any basic effect on antigen antibody reaction that produces the beneficial results in acute asthma There have been accounts of acute attacks occurring during bronchoscopy (D Abreu 1940) in which the mucosa was seen to swell up over the end of the instrument grasping it tightly and almost occluding the lumen of the bronchus This mucosal and submucosal swelling throughout bronchus and bronchiole is the main obstructive element in acute asthma rather than any muscle spasm Its reduction on steroid therapy with the opening up of the airway is the reason for the marked relief of symptoms The cause of the reaction whether allergic infective or psychogenic makes little difference as regards tissue response to steroid therapy for given a large enough dose response will usually occur in all types It is of interest that where hay fever and asthma co exist the latter usually responds better than the former

There are certain precautions needed regarding steroid therapy in asthma but these are self evident and have been emphasized in the past

Selection of patients for therapy

The trend today is to give steroid therapy to patients with severe status

in infancy as a most intractable form of infantile eczema a remission at the end of the second year usually lasts until adolescence when other allergic manifestations such as hay fever or asthma are apt to develop Response to steroid therapy is often rapid and dramatic symptoms and signs improving within 24–48 hours but careful treatment of the patient as a whole is all the more indicated A return of itching is an early sign of relapse and an indication that the dose is inadequate

Pillsburg and Urbach gave 200 milligrams of cortisone daily for 4 days and then reduced the dose by 25 milligrams every 3–4 days until a level of 100 milligrams daily was reached As this was often the critical dose reduction subsequently was by only 12 5 milligram decrements at a time Relapse rapidly set in with more rapid reduction In at least half their patients the eczema was satisfactorily controlled with a daily dose of 25–75 milligrams They did not aim at or attempt complete suppression of the condition Prolonged therapy they noted was often necessary

Osler's (Henoch Schonlein) purpura (anaphylactic (allergic) purpura)

There is relatively little in the literature regarding steroid therapy in this disorder (Stefanini *et al* 1950 Wooley 1952 and Kugelmass 1951) but it seems well worth a trial in severe cases not responding to other measures

Drug reaction

In cases of suspected drug reaction all drugs should if possible be discontinued unless there is clear evidence that only one is responsible The distinction must be made between simple hypersensitivity where a patient reacts excessively to a given dose of a drug without being allergic to it and a true allergic reaction where previous exposure to a drug has sensitized the patient irrespective of the dose The true allergic response may occur as skin reactions of various kinds blood changes (for example agranulocytosis and thrombocytopenia) or asthma or alimentary upset but the skin is the commonest site One of the commonest drug allergies is the penicillin reaction but reactions to streptomycin sulpha drugs iodine and a host of other substances also occur Serum sickness is still seen not infrequently Now that long acting forms of penicillin and other drugs are used it has to be remembered that if allergic reactions occur they may persist for some time because of persistence of the allergen in the patient s tissues at the site of injection This has to be taken into consideration in treatment The penicillin reaction may be taken as the best example of true drug allergy Sensitized by a previous course of therapy the patient may immediately react to further administration commonly with urticaria but also with a variety of skin reactions including exfoliative dermatitis or angioneurotic oedema

Harvey (1954) noted in such cases rapid improvement within 2 hours of giving a large dose of cortisone He advised an initial dose of 100 milligrams followed by 50 milligrams 4 hourly for 6 doses then 25–50 milligrams 6-hourly for a further 4 doses In very severe conditions he shortened the interval between initial doses to 2 hours Corticotrophin prednisone or prednisolone can be used similarly in appropriate dosage

Exfoliative dermatitis

This inflammation is not infrequently a manifestation of drug intolerance It was not uncommon some years ago as a complication of gold or arsenical therapy

THE PARARHEUMATIC DISORDERS

The aetiology of these diseases remains unknown Hypersensitivity is believed to play a part in at least some cases of polyarteritis and certain workers have considered that systemic lupus erythematosus is due to hypersensitivity to bacterial products or other agents (Stokes 1932 Rich 1946 Mortensen and Gormsen 1952) Talbott and Ferrandis (1956) found an impressive incidence of sensitivity to drugs vaccines and sera in these cases but considered that convincing proof had not been forthcoming Other workers had not found such evidence and held contrary views (Baehr and Levitt 1951 Baggenstoss 1952) The fact that hydrazine ophthalazine is capable of causing a lupus like disorder with the Hargraves cell occasionally present is now well recognized Whatever the truth of this matter the so called collagen disorders are considered here in the light of their therapeutic response to corticotrophin and cortisone and its analogues

Systemic (disseminated) lupus erythematosus

Systemic lupus erythematosus may appear in a variety of disguises occasionally resembling rheumatoid arthritis rheumatic fever bacterial endocarditis or a blood dyscrasia in any form it is a serious and frequently incapacitating disorder and for this reason steroid therapy is approved for its treatment The disorder like all so called collagen diseases may wax and wane relapse and remit time alone will show whether expectation of life is prolonged by cortisone and corticotrophin There is no doubt of the efficacy of symptomatic control by these agents and that in most cases they make life again livable Patients with systemic lupus erythematosus used commonly to die of intercurrent infection and occasionally of renal insufficiency The position is now reversed The steroid treated patient now commonly dies of his renal condition over which such therapy has no control Systemic lupus erythematosus though ultimately fatal may last for many years Ben Ascher (1951) for instance reported the case of one patient who survived over 23 years though this was before the diagnostic Hargraves cell had been discovered According to Jessar *et al* (1953) 80 per cent of patients die within 5 years of the onset of symptoms Before steroid therapy became available death within one year was not uncommon Dubois (1956) found the median duration of life of 59 untreated or inadequately treated patients was 24 months but in 138 patients adequately treated less than 10 per cent had died in the same period Although more proof is needed it seems likely that steroid therapy has improved the prognosis in this serious condition

Treatment

In the treatment of disseminated lupus erythematosus the dose and type of drug used must depend on the individual case the aim being to effect adequate suppression of the symptoms All cases need careful supervision for intercurrent infections can occur in suppressed form with fever which could be misinterpreted as a sign of relapse of the lupus The Hargraves (LE) cell though it may become reduced in numbers does not always disappear on treatment The white cell count usually remains low Over dosage side-effects may be inevitable for the effective suppressive dose is often over 75 milligrams of cortisone daily In patients with cardiac decompensation prednisone or prednisolone are preferred as having less salt

asthmaticus when all orthodox measures including injections of aminophylline and adrenaline have failed or when the patient is known to be adrenaline and aminophylline resistant Such treatment is given to cover the period of emergency of the acute episode and it is gradually discontinued subsequently when the attack has subsided Continuous day to day therapy for chronic asthma is much less popular in Great Britain and only used in exceptional cases There is the real risk of superadded infection at high dose levels and not all such infections are sensitive to the therapeutic agents at present available These cases are by no means uncommon and though long term antibiotic treatment may be given parallel with long term steroid therapy such patients need very close watching *and may be difficult to control Continuous corticotrophin therapy carries* the disadvantage of daily injections and West (1956) in similar long term studies in rheumatoid arthritis found allergic reactions with failure to respond further to the particular preparation of corticotrophin not uncommon In severe status asthmaticus not based on bacterial infection short term treatment of the adrenaline aminophylline resistant patient remains useful and is orthodox therapy in suitably selected cases (Pearson 1955) If effective antibiotic therapy is available concurrent infection is not an absolute contra indication

Davies and Williams (1955) reported results of steroid therapy in 44 chronic asthmatics and 6 chronic bronchitics In the asthmatics 87 5 per cent of 57 courses of therapy gave good results the temporary relief afforded lasting on an average 30 days continuous therapy in these severely intractable asthmatics was unnecessary in half the cases The period of remission after such courses varied from 1 to 240 days and 43 per cent had remission for over 3 weeks Long periods of freedom from attacks after courses of therapy is likely to be due to natural remission (Savidge and Brockbank *1954)* Ball (1954) in a blind controlled trial found 5 out of 6 cases of chronic asthma modified or much improved by corticotrophin In only 2 of 7 cases given an inert control substance was improvement seen Felix Davies and Westlake (1956) on the other hand found no evidence of any beneficial effect in a controlled study of 24 cases of chronic bronchitis in exacerbation every other case receiving 60 milligrams cortico trophin gel daily in addition to antibiotic therapy It should be mentioned that Foulds *et al* (1955) and Herxheimer and McAllen (1956) have reported some success in treating cases of asthma and nasal allergy with inhalations of hydrocortisone powder

Dosage

For treatment of the acute prostrating case of status asthmaticus the correct dose of cortisone corticotrophin prednisone or prednisolone is the one which works (Hart 1953) In some cases reported as resistant to therapy it seems likely that dosage was inadequate In a crisis dosage may have to be stepped up considerably above the usual level before therapeutic response occurs then reduction to a safer dosage level may be made In most severe cases 100 milligrams intramuscularly daily of corticotrophin 200–300 milligrams of cortisone or 40–60 milligrams of prednisone or prednisolone by mouth in divided dosage will prove a satisfactory initial dose then dosage may be reduced gradually as the condition comes under control In very severe cases initial dosage may have to be pushed even higher Rose (1954) has observed that it seems fairly certain that the duration of treatment and total dose of steroid given bear no direct relation to the length of remission produced The treatment is entirely suppressive and the duration of effect will depend essentially on the state of the underlying clinical condition

not uncommonly occur shortly before the symptoms and signs of polyarteritis Whatever the truth about hypersensitivity there is no doubt that steroid therapy suppresses the inflammatory features and is an essential form of treatment

Diagnosis

The symptoms are general and local fever and systemic upset may be unaccompanied by localizing signs for some months though the latter usually occur later They may be neurological cardiovascular renal or gastro intestinal Like systemic lupus erythematosus polyarteritis is frequently misdiagnosed commonly as rheumatoid arthritis or rheumatic fever The importance of accurate diagnosis is obvious *One should always be suspicious of a case of rheumatoid arthritis* if there are visceral manifestations or major systemic upset with high fever

Treatment

Whilst it is an essential principle to make an accurate diagnosis before starting therapy it is a fact that in polyarteritis one is often not able to do so in the earlier stages of the condition On two occasions with a provisional diagnosis of polyarteritis the author has held his hand and awaited more definite proof only to see the patient develop rapidly a fatal malignant hypertension One now prefers to institute treatment in such cases rather than run the risk of rapid fatal progression even though the diagnosis may only be provisional and unconfirmed by biopsy

Steroid therapy will usually control the symptoms of polyarteritis In the early stages of treatment suppression of fever and increase in appetite are often dramatic No one particular form of steroid therapy is to be preferred in each case the decision must be made on its merits because not all cases respond Relapse can occur on lowering the dose too rapidly Renal involvement can develop on therapy as it does without treatment

Cranial arteritis

Unaccompanied by the generalized symptomatology seen in polyarteritis and occurring in an older age group the main danger with this condition lies in its producing blindness Steroid therapy is useful and eases the severe temporal headache and other symptoms but will not in the usual dosage with certainty prevent amaurosis Nevertheless unless there are clear contra indications it should be used where symptoms are severe and prostrating and uncontrolled by simpler measures

Scleroderma and dermatomyositis

In these disorders steroid therapy does no more than produce partial relief of symptoms There is no evidence that it can induce remission or effectively alter the natural course of the disease Zion *et al* (1955) in a study of 14 patients treated with cortisone or corticotrophin obtained their best results in early cases of less than 6 months standing and in whom there was no peripheral vascular disturbance Nine patients obtained only slight improvement Nevertheless steroid therapy often does produce sufficient relief from distressing symptoms to make it worth while Appetite increases fever lessens or disappears the patient feels more supple and better in health and usually asks for the treatment to continue

retaining action If symptoms lessen dosage can be reduced accordingly and if complete remission occurs the drug may be gradually withdrawn With the present range of effective antibiotics co existent bacterial infection even tuberculous is no longer an absolute contra indication to steroid treatment Indeed according to Talbott and Ferrandis (1956) it has been noted repeatedly that antibiotics in the combined disease are ineffective unless steroids are given concurrently Pulmonary tuberculosis (Johnson and Davey 1955) and pneumococcal septicaemia (Hart 1955) have been controlled without discontinuing corticotrophin and cortisone therapy in a severe case steroid therapy should never be withdrawn unless intercurrent infection cannot be controlled without so doing Steroid therapy does not prevent renal involvement in systemic lupus erythematosus but Dubois *et al* (1952) reported restoration of function in selected patients with disappearance of proteinuria and nitrogen retention Usually however renal involvement advances relentlessly in spite of adequate symptomatic control by steroid therapy In a recent study of 10 patients treated with prednisone or prednisolone Bollet Segal and Bunim (1955) noted that renal abnormalities proteinuria haematuria cylindruria and azotaemia improved only in those cases in which they had increased or appeared during an acute exacerbation of the disease which was usually accompanied by fever and dehydration Serious renal disease was not influenced by the drugs and 2 of their patients died in uraemia

Occurrence of epileptiform seizures

Epileptiform fits and mental derangement may occur as part of the disorder If they occur on steroid therapy they are probably unrelated to it Indeed Russell Haserick and Zucker (1951) observed a definite reduction in epileptiform seizures during steroid therapy

Polyarteritis (periarteritis) nodosa

Polyarteritis is a disease with many and varied clinical manifestations caused essentially by widespread inflammatory changes in the walls of the small arteries and arterioles though the veins and capillaries may also be affected In the opinion of many the results of steroid therapy in this condition are as good as if not better than those in most of the other collagen disorders

Hypersensitivity

Since the early recorded cases of polyarteritis hypersensitivity has often appeared to play a part Gruber (1925) in a review concluded that the tissue changes represented a generalized body response which was most probably associated with hypersensitivity Kline and Young (1935) and many others since have associated this disorder with allergy and a variety of substances have been indicted as causative antigens the sulphonamides in particular The subject has been well reviewed by Talbott and Ferrandis (1956) who pointed out that there is little real evidence of a causal relationship in many of the cases quoted though they consider antibiotics the exception they state that the mass of evidence concerning antibiotics and the incidence of polyarteritis in human beings appears to support a hypersensitivity pathogenesis In reviewing the records in the literature of 395 cases Boyd (1950) noted that an acute infection had often appeared early in the course of the disease others have commented that acute upper respiratory infections

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stretching the pelts to a different degree prior to fixation or with a different time of fixation may obtain slightly different results

It is clear from these results that the ovary is necessary for the induction of the allometric phase but the mechanism involved and the reason why allometric mammary growth begins when it does is not yet fully understood In this connection Silver (1953b) found that the mammae of suckling rats were unresponsive to oestrogen administration between the eleventh and nineteenth days unless a crude extract of ox anterior pituitary was administered at the same time Apparently the pituitary synergism necessary for complete mammary growth was lacking in these young rats It is possible that the pituitary was not sufficiently mature or that the synergistic hormones were inhibited by some factor in the maternal milk A milk borne inhibitor seems unlikely since Flux (1954a) found that weaning at 15 instead of the normal 21 days did not affect the relative growth rate of the mammae of mice However he also found the mouse to be different to the rat for whilst its mammae were unresponsive to oestrogen between the tenth and fifteenth days of life (though oestrogen and pituitary extract together were effective) they responded to oestrogen at the fifteenth day

Experimentally Induced Growth

Ovarian hormones and mammary growth

In animals

Many researches have established the primacy of endocrine factors in the control of mammary growth and the conclusions from earlier work have been that whilst treatment with oestrogen could evoke full duct development such treatment in many species was incapable of causing development of a normal lobule alveolar system For full alveolar development combined treatment with oestrogen and progesterone was necessary in many species However there are species differences in the mammary response to these ovarian hormones In fact experimental animals can be placed in three broad categories (1) where oestrogen alone in physiological doses induces relatively little alveolar growth as in the mouse rat and rabbit (2) where without progesterone it causes extensive lobule alveolar growth (that is substantial mammary development) as in the monkey guinea pig cow and goat and (3) where in the absence of progesterone it induces only slight growth of the mammary duct system as in the dog (*see* review of literature Mayer and Klein 1948 Folley 1952a)

Rat response to oestrogen—In the first of these categories some indication of the minimum levels of oestrogen which give mammary growth in the rat was obtained by Silver (1953a) who was able to mimic the normal relative rate of mammary duct growth by injection of 0 1 microgram of oestradiol dipropionate on alternate days from the twenty first day of life (40 gramme rat) and then increasing the dose stepwise with the body weight She also demonstrated that folic acid is probably concerned with the growth response of mammary ducts to oestrogen since the folic acid antagonist aminopterin decreased the relative growth rate in response to injected oestrogen (Silver 1954)

Later Smith (1955) studying the quantitative interaction of oestradiol 17β and progesterone on mammary growth in the immature ovariectomized rat found that

CHAPTER 16

HORMONAL FACTORS IN BREAST DEVELOPMENT AND MILK SECRETION

J S TINDAL AND MARY L MCNAUGHT

DEVELOPMENT OF THE MAMMARY GLAND

NORMAL GROWTH

UNTIL comparatively recently work on experimental induction of mammary growth had involved mainly qualitative and subjective techniques (*see* review by Folley 1952a) Lately more interest has been shown in quantitative and objective methods It has been the general belief that the mammary gland if it grows at all grows only slightly until puberty but quantitative studies on the rate of increase in mammary gland area in relation to the rate of enlargement of the body surface as a whole show that this at any rate is not the case in the rat and mouse (*see* below) or as has been known for some time in the monkey (Folley Guthkelch and Zuckerman 1939) These studies have provided new information about the time relationships of mammary development

Cowie (1949) found that in the female hooded Norway rat the total mammary area increased isometrically (that is at the same rate as the body surface) from birth until about the twenty first day when if the ovaries were intact a phase of positive allometric growth (that is a higher rate of growth than the body surface) set in (α = 3 0) * In animals ovariectomized at the twenty first day isometric growth (α = 1 0) continued Silver (1953a) confirmed these results later and also found that ovariectomy on the tenth day of life was followed by a phase of allometric growth (α = 1 45) This might be due to the action of mammogenic steroids secreted by an adrenal cortex over stimulated by a pituitary released from ovarian restraint Flux (1954a) studied the change over from isometric to allometric mammary area increase in mice (Strong s CHI strain) with similar results except that for the allometric phase beginning at about the twenty fourth day α = 5 2 This indicates that in the CHI mouse the mammary duct system grows much faster in relation to the body surface than it does in the hooded Norway rat In the latter the onset of the allometric phase precedes vaginal opening and the initiation of oestrous cycles by a good many days that is not so in the CHI mouse in which oestrous cycles begin at about the twenty eighth day only a few days after the start of the allometric phase of mammary growth

When considering these experiments on allometric growth in rats and mice it must be noted that this work was carried out in our laboratory and under our conditions Workers in other laboratories employing slightly different methods for example

* α denotes the constant of allometry or equilibrium constant For terminology of relative growth and other information *see* Huxley and Teissier (1936)

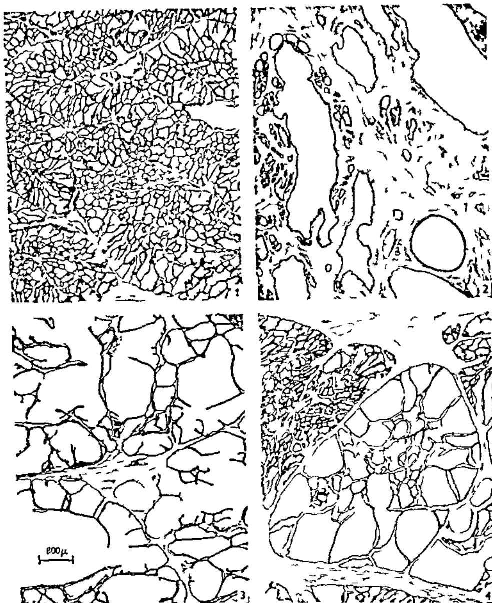


FIG 5—Alveolar tissue from goat mammary glands experimentally developed by the following treatments (1) 0 5 milligram hexoestrol and 70 milligrams progesterone daily for 150 days The alveoli are uniform and compact (2) 0 079 milligram hexoestrol daily for 140 days The field contains only a few scattered alveoli (3) 0 025 milligram hexoestrol daily for 140 days Note the extreme cystic condition of the alveoli (4) 0 25 milligram hexoestrol for 140 days The field shows two adjacent lobules one consisting of cystic alveoli and the other of alveoli of nearly normal size (*From Benson Cowie Cox Flux and Folley* 1955 *by courtesy of the Editor J Endocrin*)

oestrogen by itself stimulates not only duct but also lobule alveolar growth Progesterone alone was without effect but it potentiated the action of oestrogen on the lobule alveolar system *The degree of potentiation was directly related to* the dosage of the steroids concurrent administration was necessary for synergism to occur Smith considered that doses of 1 0 microgram of oestradiol and 3–5 milligrams of progesterone (a ratio of 1 3 000–5 000 by weight) were near optimum

Kirkham and Turner (1954) also found the oestrogen progesterone ratio for optimal mammary growth in the spayed rat to be 1 5 000 In the spayed mouse a daily dose of 0 01 microgram of oestrone doubled the total mammary area over that of spayed controls (Flux 1954a) but higher doses (0 055 microgram daily) were required to attain the total mammary areas of comparable intact mice Gardner *et al* (1935) in the mouse noted stunted duct systems from stimulation with excessive oestrogens this has since been found too in the rat rabbit and monkey (*see* Folley 1952a) Recently *Yamamoto and Turner (1956) by using both subjective and objective methods in the* rabbit have obtained an optimal oestrogen progesterone ratio for mammary growth of 1 67 (by weight) This indicates that the synergistic ratio of oestrogen progesterone in the rabbit differs from the higher ratios as observed by other workers in the mouse (Elliott and Turner 1953) rat (Kirkham and Turner 1954) and dog (Trentin *et al* 1952) Earlier work indicated that oestrogen could cause complete mammary growth in the guinea pig but Benson *et al* (1957) with quantitative methods found that oestrogen combined with progesterone gave the best mammary development in this species The optimal oestrogen progesterone ratio lay between 1 20 and 1 100 *In this species the necessity for progesterone was also noted by Smith and Richterich* (1956)

Oestrogen and the ruminants—Into the second category fall the ruminants of economic importance in which oestrogen alone induces not only duct growth but also considerable lobule alveolar development Using special techniques (devised by K C Richardson) for quantitative study of the structure of the goat udder Cowie *et al* (1952) showed that in spayed virgin goats udders grown by hexoestrol alone exhibited a variety of histological abnormalities some of which had been described first by Mixner and Turner (1943) Of these probably the most sig nificant functionally was a deficiency in the total alveolar surface area

Benson *et al* (1955) in confirmation of their earlier work (Cowie *et al* 1952) showed *that the histological abnormalities previously found in glands developed with relatively* high oestrogen dosage and also to some extent with progesterone and high oestrogen dosage for example cystic alveoli papillomatous epithelial outgrowths and immature lobules did not occur when oestrogen at a lower dose level and progesterone were given (0 5 milligram hexoestrol and 70 milligrams progesterone daily for 150 days that is 1 140 by weight) The alveolar tissue was of fairly uniform porosity (the index of porosity being defined as the alveolar surface area per unit volume of tissue) and the total alveolar surface areas of half udders removed from these experimental goats at the peak of lactation gave a high correlation with the milk yields of the half *udders*

In man

No detailed study has been carried out of the relative roles of oestrogen and *progesterone* in human mammary development although McBryde (1939) has

to induce the formation of alveoli and can also induce secretion following alveolus formation (Mizuno *et al* 1955) The last named workers concluded that the observed responses could be attributed to the action of prolactin alone

Adrenal cortex

Experiments with adrenalectomized animals have failed as yet, to indicate that the adrenals do play a part in mammary development but the administration of adrenal steroids on the other hand has yielded more positive results

Deoxycorticosterone acetate (DOCA) has been found to stimulate mammary duct growth in male mice (van Heuverswyn *et al* 1939) in the monkey (Speert 1940) and in the guinea pig (Nelson *et al* 1943) although negative results were reported by other workers (*see* Folley 1952a for review) DOCA synergized with oestrogen in causing mammary duct growth in spayed mice (CHI strain) while DOCA given alone caused stunted duct growth in about half the mice to which it was given (Flux 1954b)

As regards the 11 oxygenated corticoids cortisone and cortisol inhibited mammary duct growth in intact and oestrogen treated spayed mice (Flux 1954b) but in contrast to these results Selye (1954a) showed that in female rats cortisone and cortisol can both produce marked mammary gland development and secretion in adrenalectomized ovariectomized animals simultaneously treated with small doses of oestradiol while if the adrenals were intact ACTH was as effective as cortisol (Selye 1954b) Selye s findings have been confirmed for the intact female rat by Johnson and Meites (1955) Although these results are extremely interesting it must be noted that in view of the previous reports on the lack of effect of adrenal ectomy on mammary gland development their physiological significance must at present be regarded as not clear Since during stress excessive amounts of cortisol and allied glucocorticoids are produced by the adrenals Selye (1954a) suggested that perhaps stress can affect the mammary gland through production of corticoids thus affording an explanation for the occasional clinical case of breast development after extensive skin burns or of the sudden induction of lactation by acute exposure to stress

Thyroid

There is no evidence that the secretions of the thyroid gland are essential for mammary growth This has been convincingly demonstrated by Chen *et al* (1955) who obtained lobule alveolar mammary growth in hypophysectomized adrenalectomized gonadectomized rats whose thyroids had been ablated by surgery or ^{131}I or both and which received oestrone progesterone prolactin and STH daily for 1 week Further injection of prolactin and STH plus cortisol for 1 week evoked an abundant secretion of milk

Other recent studies by various workers lead to the general conclusion that in the rat hypothyroidism leads to enhanced development of alveoli and often but not always of the duct system as well in response to oestrogen and progesterone while the administration of thyroxine has the opposite effect In the mouse on the other hand hypothyroidism seems to inhibit mammary development while mild hyperthyroidism stimulates it As Trentin *et al* (1948) suggested it is possible that the explanation lies in the relative thyroid secretion rates in the two forms

shown that breast enlargement can be evoked by percutaneous inunction of natural oestrogen This technique however does not appear to have been much used clinically and therapeutically in mammary under development Recently an attempt has been made by Foss (1956) to initiate lactation in a castrated male transvestist He was given an implant of 500 milligrams of oestradiol and 10 months later a further 600 milligrams of oestradiol followed by daily injections of oestradiol dipropionate and progesterone for 6 weeks Immediately after withdrawal of this treatment 22 9 milligrams of prolactin were injected daily for 3 days but without effect After a second month of treatment with oestradiol and progesterone daily he was given combined injections of prolactin and somatotrophin for 4 days suction with a breast pump being employed 4 times daily On the fourth and fifth days a few drops of colostrum were expressed from the right nipple There is a possible application here of modern hormone knowledge to man and further trials would be of interest

The anterior pituitary hormones

In recent years new light has been thrown on the role of the anterior pituitary hormones in mammary gland development (Nelson 1951 1952 1954 Lyons 1951 Lyons *et al* 1953) There now appears to be no necessity for the mammogen theory (put forward by Mixner and Turner 1943 Trentin and Turner 1948 and criticized by Folley 1947a and 1952a) as complete mammary development typical of late pregnancy can be obtained by giving hypophysectomized rats oestrogen progesterone prolactin adrenocorticotrophin (ACTH) and somatotrophin (STH) and by altering the hormonal treatment it has been possible to obtain lactation by *known* hormones The ACTH was given to restore to a semblance of normal the physiological state of the hypophysectomized animal but it would appear that STH is important *per se* in mammary development (Lyons *et al* 1955)

In these experiments the prolactin could be replaced by one 12 day rat placenta daily suggesting that in the later stages of pregnancy at least in the rat the placenta can assume the role of the pituitary in secreting prolactin or a substance with the same luteotrophic and mammogenic effects (Ray *et al* 1955) A similar feat of producing complete mammary development followed by lactation has been achieved recently in the hypophysectomized male rat (Lyons *et al* 1955)

The mammary growth promoting effects of oestrogen and progesterone in intact animals are inhibited after hypophysectomy (*see* Folley and Malpress 1948a) but if insulin is administered together with oestrogen and progesterone to hypophysectomized rats the mammary gland becomes responsive to the action of ovarian hormones (Ahren and Jacobsohn 1956) This finding is in harmony with the fact that liberation of insulin into the blood is reduced after hypophysectomy in rats (Randle 1955a) that STH and prolactin stimulate the responsiveness of the mammary gland to ovarian hormones in the hypophysectomized rat (Lyons 1951) and that STH and possibly also prolactin may enhance the secretion of insulin from the pancreatic islets (Milman *et al* 1951 Foà *et al* 1955 Lukens and McCann 1955) It is also of interest that in the ovariectomized rabbit pretreated with oestrogen if the duct system is rudimentary or only slightly developed prolactin administered intraductally seems to induce duct formation while in glands with a moderately developed duct system but no alveoli prolactin seems

mechanism The idea that the mammary gland itself might become insensitive to the action of prolactin has recently been investigated by Meites and Sgouris (1953 1954) These workers showed that in gonadectomized rabbits after mammary gland growth had been effected by administration of oestrogen and progesterone initiation of lactation could be induced by prolactin alone or prolactin in conjunction with either oestrogen or progesterone but not in the presence of both Subsequently they showed that if the dose of prolactin was sufficiently high initiation of lactation could take place in the presence of both oestrogen and progesterone

These findings have led Meites to modify the original Meites Turner conception of lactogenesis being brought about solely by increased secretion of prolactin In a review of the situation Meites (1954) put forward the suggestion that lactation does not occur in pregnancy not only because of low concentrations of circulating prolactin but also because of the combined action of oestrogen and progesterone stimulating growth of the mammary gland and making it refractory to prolactin stimulation That pregnancy and lactation can co exist he attributes to the fact that the prolactin level in the blood of the lactating animal is sufficiently high to overcome the increasing levels of oestrogen and progesterone due to advancing pregnancy The concession that the mammary gland under the influence of oestrogen and progesterone may play a positive role in inhibiting lactation during pregnancy does to some extent reconcile the Meites Turner and the Nelson theories

Folley (1955a 1956) has now put forward his later views that (a) low circulating levels of oestrogen activate the lactogenic function of the anterior pituitary while higher levels inhibit lactation as was postulated in the double threshold theory of Folley and Malpress (1948b) (b) lactogenic doses of oestrogen may be rendered inhibitory by suitable doses of progesterone this being the normal inhibitory influence during pregnancy (c) the fall in the ratio of progesterone oestrogen at parturition removes the inhibition which is replaced by the positive lactogenic effect of oestrogen acting unopposed Experimental evidence for these theories has mainly been obtained in small animals and must be applied to other species with care Cowie *et al* (1952) however did note an antilactogenic effect of progesterone in virgin goats treated with oestrogen to induce lactation

How far do these observations fit in with the known facts of human lactation? In an interesting article on abnormal lactation in women Foss and Short (1951) review the literature on galactorrhoea and attempt to explain the abnormalities in terms of these current theories

Biochemistry of lactogenesis

Much interest has been taken in recent years in the mechanisms whereby hor mones regulate the metabolism of tissues and particularly in the dramatic changes brought about in mammary gland tissue at parturition by the hormone influences described above Sutherland (1955) points out the difficulties in studying such regulatory mechanisms in the intact animal since they are so integrated that when a hormone imbalance is created it is difficult to say whether an effect is primary or secondary One turns therefore to *in vitro* experiments with tissue preparations of various types but it is first essential to know what changes occur within the gland *in vivo*

in the normal rat it being somewhat above the optimum for mammary growth while in the mouse the opposite is the case

INITIATION OF LACTATION

Hormonal control

A problem which has long intrigued endocrinologists is that of the hormonal mechanisms necessary to convert the mammary gland developed into a potential secretory organ during pregnancy into an active secretory organ at parturition for although some formation of milk may occur during pregnancy (*see* Folley 1952a) copious milk secretion does not normally take place until after parturition

Since the discovery by Stricker and Grueter (1928) of the lactogenic effects of anterior pituitary extracts and the subsequent isolation of prolactin by Riddle *et al* (1933) there has been much discussion as to whether this hormone is the only one concerned in lactogenesis and how its secretion is controlled That prolactin does have a direct effect on the mammary epithelium was suggested by the experiments of Lyons (1942) who showed that prolactin injected directly into the galactophores of rabbits with prepared mammae evoked pronounced formation of milk in the lobules of the gland served by the injected galactophores These experiments were later confirmed by Meites and Turner (1948) Mizuno *et al* (1955) and at the National Institute for Research in Dairying by Bradley and Clarke (1956) Since in these experiments the rabbits were not hypophysectomized the possibility of other anterior pituitary hormones being involved cannot be excluded Furthermore the fact that injection of purified prolactin has failed to initiate lactation in hypophysectomized animals whereas unfractionated anterior pituitary extracts are effective has suggested to some workers that lactogenesis results from the action of a complex of lactogenic hormones (*see* Folley and Young 1941) Folley (1952b) however concedes that prolactin is the limiting factor in most experimental situations

Inhibition of lactation during pregnancy

Of recent years much speculation has occurred as to how the lactogenic effect of prolactin is held in check during pregnancy Writing on the subject in his article on lactation in Modern Trends in Obstetrics and Gynaecology Folley (1950) discussed the theory put forward by Meites and Turner (1942 1948) that the initiation of lactation at parturition was due to an increased output of prolactin by the anterior pituitary evoked by oestrogen a stimulus which could be inhibited by progesterone This was interpreted as an indication that during pregnancy lactation in the fully developed gland was checked by the high levels of progesterone in the blood which were keeping prolactin secretion at a minimum Earlier theories such as those summarized by Nelson (1936) had assigned to oestrogen the role of lactogenic inhibitor

Nelson (1954) reviewing the subject at the Symposium on Lactation held in Montreal drew attention to the fact that he had originally (Nelson 1937) suggested that the inhibitory influences of oestrogen worked in two ways (1) by suppressing either production or release of prolactin and (2) by direct action on the mammary gland, making it refractory to the stimulatory effect of prolactin As a result of later work (Nelson 1951 1952) this author now lays greater emphasis on the latter

thus correlating with the changes in mitochondrial respiratory enzyme activity which have been discussed The respiratory quotient (R Q) of the tissue rose from below 1 during pregnancy to around 1 5 by mid lactation These high R Q values suggest fat synthesis from the oxygen rich substrates glucose and acetate added to the medium

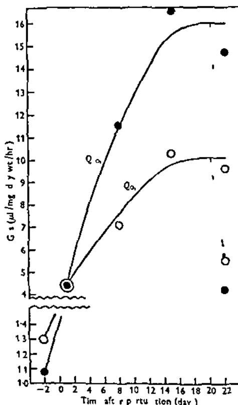


Fig 6—Gas exchange of rat mammary gland slices during late pregnancy lactation and after weaning The points at the ends of the dotted lines refer to groups of rats weaned at the twentieth day of lactation $RQ = QCO_2/QO_2$ (*Folley and French* (1949b) *by courtesy of the Editor Biochem J*)

Further studies in which labelled substrates (^{14}C glucose ^{14}C acetate and ^{3}H acetate) were added to the medium and measurement made of the activity of the fatty acids isolated from the slices at the end of the incubation period showed that the high R Q of lactating mammary gland slices was accompanied by incorporation of the carbon of these labelled substrates into the fat For rats glucose was necessary to promote incorporation of acetate carbon but with sheep acetate alone could be utilized for fatty acid synthesis (Balmain *et al* 1954)

As a consequence of their high R Q lactating mammary gland slices when incubated in bicarbonate saline solution in equilibrium with carbondioxide oxygen gas phase give rise to a steady increase in net pressure that is the composite respiration curve (CRC) has a positive slope Under the same conditions slices of tissue from pregnant animals show a CRC which is if anything slightly negative in slope

Since prolactin has been shown to have a direct action on the mammary epithelium *in vivo* it seemed possible that incubation of mammary gland slices from a pregnant animal with prolactin *in vitro* might induce respiration characteristic of lactating tissue

In vivo changes

The changes of activity in the various enzyme systems of the mammary gland during the phases of pregnancy lactation and involution after weaning have been investigated by several workers In such studies one of the problems is to find a basis on which to express activity owing to the fact that mammary gland tissue during these phases contains large and variable amounts of milk Folley and Greenbaum (1947) in studying arginase and alkaline phosphatase in rats expressed their results on moist weight corrected for milk content as estimated by the lactose content of the tissue More recently Goto and Ugami (1952) repeated the work on alkaline phosphatase and compared corrected moist weight with deoxyribonucleic acid (DNA) as a basis for expressing results Both laboratories found that when activity was expressed on a corrected moist weight basis alkaline phosphatase activity increased markedly during pregnancy and early lactation and remained high during the remainder of lactation When DNA was used as the basis essentially the same changes were observed although the differences were smaller Arginase activity increased only slightly during pregnancy and early lactation but rapidly thereafter until weaning when it decreased with even greater rapidity Greenbaum and Greenwood (1954) found a similar pattern for changes in glutamic dehydrogenase and glutamic aspartic transaminase activities On the other hand β glucuronidase activity increased during pregnancy decreased during lactation and then increased dramatically during involution Catheptic activity was also studied and found to increase steadily during pregnancy lactation and involution Glock and McLean (1954) showed that in rats glucose 6 phosphate and 6-phospho gluconate dehydrogenases enzymes of the carbohydrate direct oxidative pathway increase rapidly from parturition until involution when there is a rapid fall in activity Moore and Nelson (1952) found that the activity of succinic dehydrogenase and cytochrome oxidase in pregnant and lactating rabbits and guinea pigs increased prior to parturition the increases being maintained throughout lactation declining sharply after weaning The pattern of increases during late pregnancy and lactation and rapid decrease to resting levels after weaning found for glutamic dehydrogenase succinic dehydrogenase and cytochrome oxidase mitochondrial respiratory enzymes suggests increase in mitochondrial size or number during late pregnancy and lactation An increase in number is also indicated by the electron micrograph studies of Howe *et al* (1956) showing that whereas the transition from the resting to the secretory phase involved an approximately twofold increase in the average area of alveolar cell cyto plasm the average number of mitochondria per unit area remained constant That the enzymic constitution of the mitochondria may also change has been suggested by the work of Tuba *et al* (1955) showing that the cytochrome oxidase activity per mg N of mitochondrial preparations from lactating rat mammary gland tissue is twice that of preparations from non lactating tissue

In vitro experiments

As regards *in vitro* experiments with mammary tissue perfusion of the isolated lactating udder (for review *see* Silver 1952) has been of value in investigating the precursors for milk synthesis within the gland but not the effects of hormones on the metabolism of the tissue However Peeters *et al* (1953) reported experiments in which insulin was added to the blood perfusing a cow udder The tissue slice technique has been of value in investigating the phases of mammary metabolism The work of Folley and French (1949a b 1950) with rats confirmed by Hoover and Turner (1954) with rats and by Smith (1956) with guinea pigs showed that the oxygen uptake of surviving mammary gland slices increased sharply soon after parturition remained high during lactation and then decreased rapidly on weaning

injections of STH given in the prepartal period resulted in enhanced milk yield in the subsequent lactation period is of especial interest This work has been repeated by Brumby (1956) and by Hutton (unpublished observations) both of whom failed to confirm this carry over effect

Brumby however thinks that injections of STH earlier in pregnancy might be worth trying as some recent work of Flux (1957) indicates that STH and thyroxine may cause increased mammary duct growth in hypophysectomized mice

Attempts to detect an *in vitro* STH effect on lactating mammary gland slices have been made by the present writer (McNaught quoted by Folley 1955b) These experiments show that growth hormone added to the incubation medium causes small but demonstrable increases in R Q oxygen uptake and glucose utilization an effect which might well be interpreted as galactopoietic It is qualitatively similar to the stimulation produced by insulin in lactating mammary tissue (*see* below) Ottoway (1953) also found an *in vitro* insulin like effect when rat diaphragm was incubated in the presence of growth hormone which he attributed to liberation of insulin from the tissue For more detail the reader is referred to the article by Folley (1955b) and the ensuing discussion at the International Symposium on Hypophyseal Growth Hormone Nature and Actions

Prolactin

Prolactin was shown by the early studies of Folley and Young (for reviews *see* Young 1947 Folley and Young 1952) to have very little galactopoietic activity a finding confirmed by other workers However in recent experiments with rats hypophysectomized early in lactation Cowie (*see* review by Cowie and Folley 1955) has shown that although prolactin alone has virtually no lactation maintaining effect prolactin plus ACTH has some replacement value suggesting that the two hormones are components of an anterior pituitary galactopoietic complex Balmain and Folley (1952) and Dr T R Bradley working in the same laboratory on the *in vitro* effect of prolactin on mammary tissue slices from fully lactating rats also detected very little effect whereas in early lactation before the gland is fully active considerable stimulation was observed as already mentioned Recently Read and Moore (1956) have observed rapid increases in Co A activity when prolactin is injected *in situ* into early lactating guinea pig mammary glands suggesting that the indications of increased mammary activity in the *in vitro* experiments if indeed it is due to prolactin and not to a contaminant (*see* above) may have their counterpart *in vivo* There seems no doubt that prolactin does not have any dramatic effect on fully lactating mammary tissue *in vitro*

The identification of growth hormone as the main galactopoietic factor of the anterior pituitary does to some extent offer an explanation of the disappointing results of the use of prolactin clinically in the treatment of hypogalactia in parturient women (a subject reviewed by Voss in 1941—*see also* Robinson 1947)

Adrenal cortex

The numerous studies by Cowie and others at the National Institute for Research in Dairying (for review *see* Folley 1953) have clearly demonstrated that in rats adrenalectomy after the initiation of satisfactory lactation at parturition interferes with subsequent lactation an effect which may be overcome by treatment with

Balmain and Folley (1952) however found that the addition of purified prolactin to the incubation medium had no effect on the slope of the CRC of mammary gland slices from pregnant rats whereas with tissue from rats in early lactation the slope of the curve was increased Working in the same laboratory Dr T R Bradley has confirmed this stimulation of early lactating tissue with prolactin Further investigation showed however that the concentration of prolactin required was high and that some preparations of STH and ACTH gave a similar effect It seemed possible therefore that a contaminant of the prolactin preparation might be responsible and in a collaborative study (Bradley *et al* 1954) intermedin was indicated as a possible contaminant

It could of course be argued that if mammary gland tissue is rendered refractory to prolactin *in vivo* by the action of oestrogen and progesterone it is not surprising that prolactin added *in vitro* to tissue from a pregnant animal does not regularly give an effect Indeed, Sgouris and Meites (1953) have shown that homogenates of mammary tissue taken from rats 4 days post partum inactivate prolactin 8 times faster than a similar preparation of tissue from rats in mid pregnancy This finding they interpret as confirming their views that mammary gland is refractory to the action of prolactin during pregnancy

MAINTENANCE OF LACTATION GALACTOPOIESIS

Studies on the stimulation of established lactation (galactopoiesis) have been made by many workers with the object of throwing light on the maintenance of lactation as it seems likely that the hormonal mechanisms are closely related Such studies were initiated in many cases with a view to increasing milk production from domestic animals but they may also be of value in indicating possible treatment of hypogalactia in women

Somatotrophin

The early studies of Folley and Young with unfractionated extracts of anterior pituitary in which pronounced galactopoiesis in cows was observed suggested that the galactopoietic activity was closely correlated with the diabetogenic activity of the extracts (*see* reviews by Young 1947 and Folley 1947b) Subsequent studies have attributed the diabetogenic activity to STH (Cotes *et al* 1949b) *Accordingly purified STH was tested as a galactopoietic agent in cows* by single injection tests and shown to be active (Cotes *et al* 1949a) Confirmation of this galactopoietic effect was provided by Donker and Petersen (1951 1952) Chung *et al* (1953) Wrenn and Sykes (1953) Brumby and Hancock (1955) and Shaw *et al* (1955) for cows and Jordan and Shaffhausen (1954) for sheep

Recently Mr J B Hutton (unpublished observation) working at the National Institute for Research in Dairying found a linear relationship between the log of dose of STH from 6 25 to 200 milligrams given as single injections and milk yield in cows

Brumby and Hancock (1955) and Chung (1955) demonstrated that the effect of STH was not due to contamination by thyrotrophin (TSH) Further they observed galactopoietic effects in early lactation and no temporary depression of expected milk yield on cessation of treatment with STH further indications that thyroid active materials differ from STH in their effect on the mammary gland The observation of Shaw (1955) (*see also* Chung 1955) working with twin heifers that

Endocrine pancreas

Since lactating mammary gland uses glucose both to provide energy and carbon for synthesis of milk solids it was thought that insulin might affect lactation through its control of the blood sugar and the various pathways of glucose utilization As already mentioned lactating mammary gland slices from rats actively synthesized fat from small molecules glucose or glucose plus acetate but not from acetate alone (Folley and French 1950 Balmain *et al* 1954) The

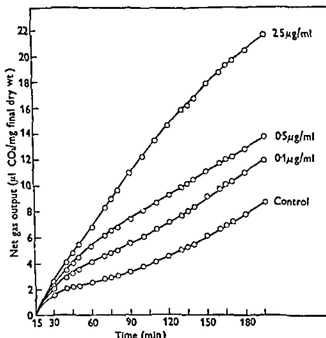


FIG 7—Effect of various concentrations of insulin on the respiratory metabolism of slices of rat mammary glands (*Balmain Cox Folley and McNaught* (1954) *by courtesy of the Editor J Endocrin*)

addition of insulin to the incubation medium very markedly increases the R Q and glucose uptake of the tissue slices and experiments with isotopes show that the rate of fat synthesis is increased (Balmain *et al* 1952) As a consequence of the increased R Q the slope of the CRC is increased an effect which has been used in developing an *in vitro* bioassay for insulin (Balmain *et al* 1954) These insulin effects were observed with mammary gland slices from lactating rats but not from rats in late pregnancy or after weaning

In this work an interesting species difference was also found Whereas glucose alone but not acetate alone was utilized for fat synthesis by rat mammary gland slices acetate alone but not glucose alone was used by sheep udder slices (Folley and French 1950) an interesting observation in view of the fact that in ruminants the blood sugar level

cortisone and deoxycorticosterone Later Cowie and Tindal (1955) compared the ability of aldosterone cortisol 9 α fluorohydrocortisone and 9 α chlorohydro cortisone to maintain lactation in such rats and found that chlorohydrocortisone (100 micrograms daily) gave virtually complete maintenance whereas 100 micro grams fluorohydrocortisone 100 micrograms hydrocortisone or 50 micrograms aldosterone were much less effective

Mammary gland tissue was taken from the animals at the end of this experiment and the respiratory activity measured *in vitro* (McNaught unpublished) Tissue from the chlorohydrocortisone treated animals showed similar respiratory activity to the sham operated control group whereas that of the other groups was significantly lower This finding would seem to indicate that respiratory activity measured *in vitro* is a fairly reliable index of the functional activity of the gland *in vivo*

The effect of chlorohydrocortisone and fluorohydrocortisone on the survival and growth of adrenalectomized rats was also studied by Llaurado (1955a) He found that the halogenated corticoids were comparable to aldosterone in activity Llaurado (1955b) extended his studies to determine whether these synthetic corti coids would allow pregnancy and subsequent lactation to proceed normally in adrenalectomized rats His experiments clearly showed that chlorohydrocortisone was effective in this respect Unfortunately insufficient fluorohydrocortisone was available to complete the work

The effect of various adrenal steroids on lipogenesis in lactating mammary gland slices has been studied by McNaught *et al* (1955) Antagonism was found between insulin on the one hand and deoxycorticosterone corticosterone and cortisone on the other insulin stimulating and the corticoids inhibiting These results illustrate again the metabolic antagonism between insulin and the adrenal corticoids which *in vivo* is expressed by the antidiabetogenic action of insulin and the diabetogenic action of the glucocorticoids The inhibition of activity of mammary tissue by added steroids may to some extent explain the finding of Cotes *et al* (1949a) later confirmed by other workers that ACTH depresses the milk yield of cows

Thyroid

The well known galactopoietic effect of thyroid active materials in farm animals has been reviewed by Blaxter *et al* (1949) Folley (1950) Blaxter (1952) and Leech and Bailey (1953) The search for the peripherally active thyroid compounds (*see* Chapter 1) has brought to light several active substances of which triiodo thyronine has been tested for galactopoietic activity in cows (Bartlett *et al* 1954) and found to increase milk yield when injected subcutaneously but not when given orally so precluding its use on the farm

Clinically the use of thyroid compounds in the treatment of hypogalactia in women does not seem to have made much progress Robinson (1947) found some improvement in women treated orally with *l* thyroxine and dried thyroid Lelong *et al* (1950) and Giraud and Coignet (1953) administered iodoprotein orally and found little effect on milk yield during the first 15 days of lactation but thereafter a substantial increase took place Thus the treatment was useful in prolonging secretion but of no value in treating primary hypogalactia

factor) causing contraction of an effector mechanism in the mammary alveoli leading to increase in intramammary pressure shortly after the start of milking or suckling which expels milk from the alveoli

The milk-ejection reflex is known to occur in a wide variety of species and probably occurs throughout the Mammalia Like all reflexes it can be conditioned the classical example being in the cow in which it becomes conditioned to the auditory tactile and visual stimuli associated with the milking routine Waller (1938) recorded an example of conditioning in a lactating woman Newton and Newton (1950a 1951) considered that this reflex was an important factor in successful human lactation and that breast engorgement was caused by retention of milk in the alveoli due to the reflex failing Isbister (1954) considered that in women the reflex was essential for successful lactation and that over 95 per cent of the women studied by her with established lactation were conscious of the reflex Like other conditioned reflexes it can be inhibited by emotionally disturbing stimuli as has been shown experimentally in the cow by Ely and Petersen (1941) and Whittlestone (1951) by Newton and Newton (1948) in woman and by Cross (1955b) in the rabbit Not only are disturbing stimuli and embarrassment highly undesirable in the lactating woman but the state of mind and general mental approach of the mother to breast feeding is most important (Newton and Newton 1950b)

Evidence for the neuro endocrine nature of the milk ejection reflex

There is considerable evidence that the terminal link in the milk ejection reflex involves the action of oxytocin At the National Institute for Research in Dairying Macaulay (cited by Folley 1952b) carried out experiments to find whether milking depleted the oxytocin content of the neurohypophysis of goats She could not detect any effect of milking on the concentration of either the pressor antidiuretic or oxytocic principles in the goat neurohypophysis nor any difference between the mean values for lactating and non lactating goats Whittlestone *et al* (1952) found in the cow no evidence of depletion after milking In smaller animals (rat guinea pig and cat) Dicker and Tyler (1953) demonstrated less oxytocic activity in the posterior pituitary in lactating than in non lactating animals and this was confirmed in the dog by Van Dyke *et al* (1955) The negative results obtained in the cow and the goat are not surprising since as a result of recent work we know that the amounts of oxytocin which evoke milk ejection are extremely small

Cowie (cited by Folley 1952b) obtained definite evidence in the anaesthetized goat of milk ejection with intravenous doses of oxytocin as low as 5 milliunits (mU) although 1 unit was required to give a response comparable in duration with that obtained during normal milking Andersson (1951c) reported a similar minimum dose for the goat Denamur and Martinet (1953) obtained milk-ejection responses in the anaesthetized goat with as little as 1 mU oxytocin In anaesthetized lactating rabbits definite milk ejection responses were obtained by Cross and Harris (1952) after an intravenous injection of 5–200 mU posterior pituitary extract in the anaesthetized lactating rat hungry pups were able to obtain milk after the nursing rat had been given a single intraperitoneal injection of 250 mU oxytocin (Cowie cited by Folley 1952b) Cowie was also able to rear the young of two neurohypophysectomized rats at almost the normal rate by giving the mother an intraperitoneal injection of 500 mU oxytocin 3 times daily thus giving the young three suckling periods without this treatment the young could obtain no milk and soon died In later work (Benson and Cowie 1956)

is relatively low and volatile fatty acid level is high Further it has been shown (Balmain *et al* 1954) that sheep tissue is not responsive to insulin *in vitro* In this connection preliminary experiments by McCormick and Young quoted by Randle (1955b) suggest that small doses of insulin given to a cow are galactopoietic If these results are confirmed the lack of response in sheep slices *in vitro* may repay further investigation

Ovarian hormones

In discussing the hormonal factors involved in the initiation of lactation the theories of Meites and Turner in casting oestrogen in the role of lactation stimulator through the anterior pituitary and of Nelson who saw it as an inhibitor have been described Reports of oestrogen as stimulator and as inhibitor of established lactation have been discussed in a comprehensive review by Mayer and Klein (1949) and also by Folley (1947b) Folley and Malpress (1948b) and Cowie and Folley (1955) To account for the apparent paradox of oestrogen acting both as galactopoietic and inhibitory agent Folley and Malpress (1948b) put forward the double threshold theory

Interest in the action of oestrogens on the lactating mammary gland has centred mainly on their use in preventing or terminating lactation in parturient women a subject reviewed by Barnes (1947) Since then much conflicting evidence has accumulated on the efficacy of both natural and synthetic oestrogens in this field The question at issue is whether oestrogens given to parturient women are true inhibitors or whether termination of lactation is due to removal of the suckling stimulus provided by the baby the undoubted beneficial effects of oestrogen being due to their action in preventing painful engorgement during this period Of recent years emphasis has been laid on the synergism between oestrogen and progesterone in inhibiting lactation in experimental animals (Fauvet 1941 Barsantini and Masson 1947 Cowie *et al* 1952 Meites and Sgouris 1953) It would seem therefore that combined treatment with oestrogen and progesterone might be more efficacious than oestrogen alone in inhibiting lactation in the puerperium Indeed Romani and Recht (1948) found that concurrent administration of hexoestrol dipropionate and progesterone gave more effective inhibition of lactation in women than oestrogen alone

THE PHYSIOLOGY OF SUCKLING AND MILKING

The milk ejection reflex

The correct functioning of the milk withdrawal mechanism is just as important as milk secretion by the cells of the alveoli as without it the end result would be the same as if secretion itself had ceased The milk ejection reflex (a term recommended by Cowie *et al* 1951) known in some circles as the let down (a term severely criticized by Folley 1947c) and in medical circles as the draught has been long recognized It manifests itself by a sudden rise in the milk pressure in the gland from stimulation of the sensory nerve endings in the nipple by the suckling or milking stimulus Cathcart *et al* (1948) considered the human female nipple to be one of the most highly innervated tissues of the body The reflex was originally thought to be purely neural but it was later shown that a neuro endocrine arc was probably involved (Ely and Petersen 1941) The terminal link was believed to involve secretion of a principle by the neurohypophysis (probably the oxytocic

factor) causing contraction of an effector mechanism in the mammary alveoli leading to increase in intramammary pressure shortly after the start of milking or suckling which expels milk from the alveoli

The milk ejection reflex is known to occur in a wide variety of species and probably occurs throughout the Mammalia Like all reflexes it can be conditioned the classical example being in the cow in which it becomes conditioned to the auditory tactile and visual stimuli associated with the milking routine Waller (1938) recorded an example of conditioning in a lactating woman Newton and Newton (1950a 1951) considered that this reflex was an important factor in successful human lactation and that breast engorgement was caused by retention of milk in the alveoli due to the reflex failing Isbister (1954) considered that in women the reflex was essential for successful lactation and that over 95 per cent of the women studied by her with established lactation were conscious of the reflex Like other conditioned reflexes it can be inhibited by emotionally disturbing stimuli as has been shown experimentally in the cow by Ely and Petersen (1941) and Whittlestone (1951) by Newton and Newton (1948) in woman and by Cross (1955b) in the rabbit Not only are disturbing stimuli and embarrassment highly undesirable in the lactating woman but the state of mind and general mental approach of the mother to breast feeding is most important (Newton and Newton 1950b)

Evidence for the neuro endocrine nature of the milk ejection reflex

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750 mU oxytocin was injected twice and sometimes 3 times daily after posterior lobectomy in the lactating rat

In an investigation of women who were unable to feed their babies but who were suffering from heavy pains in breasts overloaded with milk Haeger and Jacobsohn (1953) observed that during 10 minutes suckling after an injection of 2 i u oxytocin into the mother the child obtained 3 times more milk than during the 5 minute period prior to injection This effect of oxytocin was regular in the primiparae and in 5 of 12 multiparae Four multiparae were relieved of subjective symptoms although milk yield remained low in 3 multiparae the injection had no effect Haeger and Jacobsohn concluded that milk ejection had been absent in most of these cases from a deficient final link in the neurohormonal reflex which was replaced by injections of oxytocin

Several workers have provided further evidence that the milk ejection reflex involves activation of the posterior pituitary An antidiuretic effect in water loaded lactating subjects has been observed shortly after application of the milking stimulus in the rabbit (Cross 1951) in the bitch (Kalliala *et al* 1952) in the cow (Peeters and Coussens 1950) and in woman (Kalliala and Karvonen 1951) However Cross (1951) concluded that the release of ADH vasopressin which occurs in suckled female rabbits is insufficient to exert any effect on milk ejection

Recently the posterior pituitary hypothesis has been directly confirmed by electrical stimulation of the supra optico hypophysial system Andersson (1951a 1951b) obtained milk ejection responses in the lactating sheep and goat by electrical stimulation of hypothalamic areas adjacent to the supra optic nuclei and responses were obtained in denervated udder halves and during sacral anaesthesia pointing to a terminal hormonal component of the reflex In the anaesthetized lactating rabbit electrical stimulation of the pituitary stalk region resulted in ejection of milk similar to that after intravenous injection of 50–200 mU posterior pituitary extract (Cross and Harris 1951) and similar results were obtained by electrical stimulation of the supra optico hypophysial tract (Cross and Harris 1952) It is interesting that the time lag in response to stimulation was typical of humorally mediated effects Cross and Harris (1952) also showed that electrolytic lesions could be placed in the supra optico hypophysial tract in such a way as to prevent suckling young from obtaining milk and to interfere with the response to stalk stimulation presumably after degeneration of the tract had occurred The young however could obtain milk after the injection of posterior pituitary extract On the other hand in those rabbits in which there was no disturbance of milk ejection following placement of lesions electrical stalk stimulation still evoked the milk ejection response Further evidence for the role of oxytocin in the milk ejection reflex has been obtained from studies on the mechanism of inhibition of the reflex Cross (1955b) investigating the emotional factor and disturbance of milk ejection in rabbits suckled while under forcible restraint found that in 35 of 42 experiments the milk yield obtained by the young was reduced by 20–100 per cent and that there was a correlation between the size of the reflex response and amount of milk withdrawn

Normal milk removal was associated with a reflex response corresponding to about 0 05 i u injected oxytocin half the normal yield with a response equivalent to 0 005–0 01 i u of oxytocin and less than 15 per cent of the milk yield with complete absence of any reflex milk ejection Emotional inhibition of oxytocin release was accompanied

in a few cases by a peripheral inhibitory effect in the mammary glands as shown by the failure of an injection of 0·05 i u of oxytocin to restore normal milk removal In such cases the recorded milk-ejection response to the injected oxytocin was reduced in size by an amount similar to that produced by an intravenous dose of 1 microgram of adrenaline given just before 0 05 i u of oxytocin It seems highly probable that the peripheral block occurring in these cases involved a mammary vasoconstriction due to activation of sympathetico adrenal pathways from the hypothalamus (Cross 1953 1955a)

Cross concluded that while activation of the sympathetico adrenal system can occur the main factor in the emotional disturbance of the milk ejection reflex was a partial or complete inhibition of oxytocin release from the posterior pituitary gland

The nature of the milk ejection hormone

The brilliant work of du Vigneaud who isolated the purified oxytocic and ADH vasopressin peptides of the neurohypophysis and finally synthesized them (for review *see* du Vigneaud 1956) and the biological assays carried out by Cross and Van Dyke (1953) and Van Dyke *et al* (1955) leave no doubt that the endocrine link in the milk ejection reflex is oxytocin and in an investigation of the milk ejecting activity of natural and synthetic oxytocin in women Nickerson *et al* (1954) found that both synthetic and natural oxytocin were of equal potency Supporting evidence was provided by Peeters and Coussens (1950) who observed that in the cow mild fright during milking inhibited the antidiuretic response without inter fering with the milk ejection reflex

After destruction of posterior lobe function or removal of the lobe in lactating rats the milk ejection reflex was abolished and the pups could only be reared if the mothers were injected with oxytocin (Harris and Jacobsohn 1952 Cowie cited by Folley 1952b) However when posterior lobectomized rats became pregnant a second time parturition was normal and the milk ejection reflex was sufficiently restored to enable the rats to rear their litters without the administration of exogenous oxytocin (Benson and Cowie 1956) In these animals an increase in size of the neural stalk had occurred (also observed by Stutinsky 1952 and Billenstein and Leveque 1955 after hypophysectomy in the rat) The results are in agreement with recent theories that the posterior lobe hormones are secreted in the hypothalamus and are carried down the axones of the hypothalamo hypo physial tract into the neural lobe (Scharrer and Scharrer 1954)

The contractile effector tissue of the mammary gland

In the past there has been uncertainty as to what effector contractile tissue was responsible for milk ejection Richardson (1947 1949a) found no evidence of smooth muscle fibres in close association with the mammary alveoli However the presence of myoepithelial cells (basket cells) has been recognized for a long time In the mature mammary gland these cells represent the derivation of the outer of the two epithelial layers lining the ducts in the immature gland Richardson (1949a 1949b) was able to demonstrate them clearly for the first time in the goat and in man (Richardson 1951) as flattened stellate cells lying on the outside of the alveoli between the epithelium and the basement membrane and with their processes enveloping the alveolar surface so justifying the term basket

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gland and may be interpreted as harmonizing with the view that oxytocin stimulates prolactin secretion Direct vascular connections from the posterior to the anterior lobe of the pituitary have been demonstrated in the rat (Landsmeer 1951 Daniel and Prichard 1956) so it seems possible that the anterior lobe may receive blood highly charged with oxytocin before any dilution of the hormone can occur with blood from other parts of the body If further work confirms that this interesting effect of oxytocin is mediated by the release of prolactin by the anterior pituitary then we have here a means by which two of the main phases of lactation—milk secretion and milk ejection—are integrated (for terminology of scheme *see* Cowie *et al* 1951)

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cell This was confirmed by Linzell (1952) Richardson considers that the histological appearance of these cells before and after milking agrees with the view that they contract actively during milking and Linzell (1955) has shown by direct microscopic examination of the living mammary gland of the mouse that oxytocin topically applied can cause contraction of the alveoli forcing milk into the larger ducts



FIG 8 —Tangential sections of alveoli showing myoepithelial cells closely adherent to the mammary epithelium with the intervening stroma almost completely unstained (*By courtesy of K C Richardson*)

Neurohypophysis and the maintenance of lactation

In addition to the role of oxytocin as the hormonal agent of the milk ejection reflex evidence has recently been obtained that the posterior pituitary may also play a part in the maintenance of lactation It is known that the suckling stimulus can maintain lactation and prevent involution of the mammary gland (Selye 1934) presumably because it causes release of prolactin from the anterior lobe of the pituitary Benson and Folley (1956) greatly retarded mammary involution by administering oxytocin to lactating rats whose litters had been removed at the fourth day of lactation In later work (Benson and Folley 1957) these findings were confirmed and a similar response was obtained both with a natural preparation of oxytocin free from preservative and with synthetic oxytocin ADH vasopressin only had a slight effect in preventing mammary involution In rats which were hypophysectomized at the same time as the litters were removed oxytocin did not prevent involution of the mammary gland but sham operated controls responded to oxytocin to the same extent as in the earlier experiments These results rule out the possibility of oxytocin having a direct action on the mammary

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CHAPTER 17

ENDOCRINE FACTORS IN THE AETIOLOGY AND TREATMENT OF CANCER OF THE BREAST AND PROSTATE

BASIL A STOLL

INTRODUCTION

AN IMPORTANT development in recent research was the suggestion that the initiation and growth maintenance of some tumours may be partly under endocrine control As early as 1889 Schinzinger suggested castration in treating young females with breast cancer and White in 1893 suggested castration for benign prostatic hypertrophy The investigation of the hormonal relationships of these tumours received a new impetus following the dramatic benefit achieved in carcinoma of the prostate by castration (Huggins and Stevens 1940) and later by the use of stilboestrol In the treatment of cancer hormones have the advantage of not inducing local tissue destruction unlike some of the other modalities used so that even if of no benefit they will do no great harm to the patient The outstanding difference in their mode of action is that they aim to modify the internal environment of the host and so increase the resistance of the patient to his tumour As a result cancer could come under control whatever the degree of its dissemination

It will be shown later that certain tumours particularly cancer of the breast and prostate can be regarded as hormonal in origin When malignancy is established the tumour although possessed of a certain degree of autonomy may still *retain some sensitivity to hormones and therefore is called hormone dependent* The pioneer observations of Huggins on prostatic carcinoma were possible because in the case of the prostate the normal hormone control is not complex Androgens stimulate both normal and cancerous prostatic tissue to grow, whether the source of the androgen is the testis or the adrenal cortex and modification of this control is relatively simple

There is however great difficulty in modifying the hormonal control of the breast because of the complexity of its hormonal relationships The organ is known to be influenced not only by two hormones from the ovary and by the adrenal cortex but also by at least two hormones from the pituitary and by the thyroid gland in the normal female Associated with multiple hormonal control is the tendency for decrease in secretion of one hormone to lead to over secretion of another the pituitary gonadal relationship being compared by Huggins to a *closed cycle feedback system* Thus it is well established that deficiency of the ovarian hormone due either to the menopause or castration may lead to over secretion of pituitary follicle stimulating hormone Conversely if one establishes an excess of ovarian hormone by therapeutic administration depression of pituitary secretion may result

It follows therefore—and this is a most important factor from the point of view

of cancer therapy—that imbalance of hormones achieved by whatever method will yield only temporary results As will be seen later this is the case so far in both carcinoma of the prostate and carcinoma of the breast when controlled by hormonal methods

CARCINOGENIC ACTION OF STEROIDS

The steroids of the body are based on cholesterol and it is known that the related compound deoxycholic acid can be transformed in the laboratory into methyl cholanthrene The hydrocarbons of the polycyclic aromatic group which are capable of inducing carcinoma in experimental animals include dibenzanthracene benzpyrene and methylcholanthrene It is possible therefore that carcinogenic hydrocarbons may arise from steroids in the human body and in this connexion mention should be made of the work of Dobriner *et al* (1947) who showed that adrenocortical activity is frequently disturbed in cancer patients

Lipschutz (1950) stressed that experimental oestrogenic tumours which can be induced in animals (such as uterine fibroids endometrial adenomas and pituitary tumours) develop only when oestrogen administration is continuous and at a hyperphysiological level In most of these animals on the other hand the physiological concentration of oestrogens is at a low level and is rhythmic in character varying with the sex cycle As discontinuous oestrogen concentration allows tissues to return to their normal condition at rest Lipschutz suggested that in these experiments the tissues have no hereditary abnormality but only because of abnormal concentration and timing of oestrogens do these benign tumours arise Gardner in 1941 observed that a very high dose of oestrogen may even inhibit the growth of breast tissue although small doses are stimulant

Does oestrogen tumorigenesis act via the gonadotropic or corticotropic secretion of the anterior pituitary? Can a high oestrogen level in the body lead to the secretion of abnormal steroids noted by Dobriner? Is there a body defence against oestrogen tumorigenesis? These questions remain to be answered and with regard to the last it is well known that the liver plays an important part in the inactivation of oestrogens in the body Damage to the liver either by vitamin B deficiency or protein deficiency reduces its ability to inactivate endogenous or exogenously administered oestrogen The clinical significance of this fact may be reflected in the high incidence of mammary carcinoma in Bantu males in Africa where it is possibly associated with a deficient diet In addition Biskind in 1944 suggested *that cystic mastitis and uterine myomas may be associated with vitamin B deficiency* and in 1947 Ayre noted thiamine deficiency in a high proportion of his cases of carcinoma of the cervix uteri in Canada

HORMONAL INFLUENCES IN SPONTANEOUS MAMMARY CANCER IN MICE

Much of the knowledge of hormonal control of cancer is based on experiments on mice carrying hereditary mammary adenocarcinoma It is possible to influence the development of spontaneous mammary tumours in mice by the following methods (1) changing the genetic factor (2) hormonal changes for example castration

oestrogen or testosterone administration hypophysectomy induction of hypothyroidism adrenalectomy (3) avoiding the milk factor transmitted in the mother s milk (Bittner 1936) and (4) dietary restriction for example restricted protein or calorie intake or restriction of certain amino acids or members of the vitamin B complex in the diet

Only the first two factors are discussed in this chapter

Genetic factor and the influence of castration oestrogens and testosterone

Certain strains of mice can be bred to carry almost a 100 per cent likelihood of all the females developing spontaneous mammary cancer and other strains bred to carry a minimal likelihood

In 1932 Lacassagne was able to induce breast cancer in male mice by administration of oestrogens and thus oestrogens were the first compounds originating in the body which could be shown to cause cancer In strains where the susceptibility is high mammary cancer develops spontaneously in the female when exposed to physiological doses of oestrogens Ovariectomy or the administration of testosterone before full female development delays or reduces the incidence of cancer in these strains but in 1918 Lathrop and Loeb showed this does not apply if the animals are castrated at an older age The mammary gland having then been exposed to a certain number of oestrous cycles

Hypophysectomy

Hypophysectomy reduces the rate of growth of mammary tumour transplants stated Korteweg and Thomas in 1939 and of spontaneous mammary cancer according to Gardner in 1942 Smith *et al* (1954) have shown that the administration of pituitary somatotropin to C3H mice bearing transplanted mammary adenocarcinoma leads to an increase in tumour growth not related to the increase in animal weights Schulman and Greenberg (1949) were however unable to observe similar results in A strain mice

Stimulation to breast cancer in susceptible mice by oestrogens probably requires the presence of pituitary function Lacassagne and Chamorro (1939) showed that oestrogens alone could not effect the development of breast cancer in hypophysectomized male mice In this respect mention should be made of the recent work of Lyons (1956) who showed that full growth of the breast in gonadectomized and hypophysectomized male mice requires a relatively high concentration of prolactin and a low concentration of oestrogens The addition of growth hormone and cortisone can even induce lactation Although most authorities find the anterior pituitary biologically inert when transplanted Muhlbock (1954) has induced mammary carcinoma in mice in the absence of both the milk factor and of extraneous oestrogens by the implantation of fresh anterior pituitary gland This effect may be a result of its prolactin content

Hypothyroidism

Both thiouracil administration and thyroidectomy reduce the incidence of spontaneous mammary carcinoma in mice of the C3H strain (Morris *et al* 1946) There is of course an associated anoestrus and decreased output of oestrogens from the ovary and these changes may be responsible for the decrease in mammary carcinoma noted

Long-continued thyrotropin administration reduces the incidence of spontaneous mammary cancer in mice of the *RIII* strain at the same time inhibiting their fertility (Cramer and Horning 1938) It is possible that both thyrotropin and thiouracil administration decrease cancer development partly as a result of nutritional depression

Adrenalectomy

It is well known that if animals are ovariectomized at birth the adrenal cortex can take over the hormonal function of the ovary in the development of the breast and the uterus Adrenalectomy will impair the effect of oestrogens in inducing breast cancer in susceptible strains of mice (Cramer and Horning 1939) and will decrease its incidence

The hormonal control of spontaneous mammary carcinoma in mice is summarized graphically in Fig 9 It is seen that testosterone administration castration adrenalectomy hypophysectomy and induced hypothyroidism depress the growth while oestrogen or anterior pituitary administration stimulate the growth of spontaneous mammary carcinoma

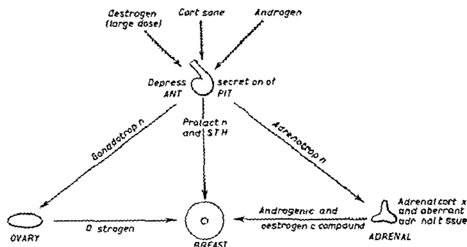


FIG 9—Hormone control in experimental animals with mammary cancer

HUMAN MAMMARY CARCINOMA

Endocrine relationships of human mammary carcinoma

Age incidence—The incidence of breast carcinoma in females reaches a peak at the age of 45 years but when allowance is made for age distribution in the population it is statistically shown that the *risk of developing breast cancer increases* proportionally with advancing age Leucutia stated in 1944 the poorest prog nosis is in cases occurring between 50 and 59 years although according to Clemmesen in 1948 and to Anderson in 1950 there is a decrease in the incidence noted in this age group These observations are shown graphically in Fig 10

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found in 55 per cent of patients with breast cancer compared with 28 per cent of the normal population Delayed menopause means a longer exposure to oestrogen concentration

The adverse effect of pregnancy on carcinoma of the breast probably depends more on vascular changes than on the oestrogen or prolactin concentration Incomplete lactation is said Adair in 1934 often associated with development of carcinoma of the breast later In these cases obstruction or irritation of the lacteals may predispose to malignancy and the habit of prolonged lactation may be responsible for the low incidence of the disease in Japan—about one fifth of that in Western countries

Hypothyroid state —The idea that patients with breast carcinoma are more *likely to be in a hypothyroid state and therefore benefit from thyroid hormone* administration was revived by Loeser in 1954 Spencer (1954) suggested that a high thyroxine level in the blood can suppress pituitary activity in the same way as do oestrogens The experimental evidence is confusing According to Chu and You (1944) small doses of thyroid extract experimentally reinforce the action of oestrogens while large doses completely inhibit ovarian activity

Scheme of hormone therapy in breast cancer

The following methods of hormone control are practised in the treatment of advanced breast cancer each method being tried for at least 2 months before being abandoned for the next on the list Radiotherapy is generally utilized for the control of the early localized manifestations hormone control being reserved till the disease is widespread With hormone therapy the average survival *following the* first recurrence is 19 months compared with only 8 months for untreated cases (Peters 1956)

Premenopausal cases

(1) Surgical oophorectomy can induce a long remission in hormone dependent cases that is in about 40–50 per cent of the total Various authors quote relief in 30–55 per cent of cases following radiation menopause but its effects are more delayed and less complete Relief lasts for a period varying from 6 to 24 months (averaging 9 months) the best results being noted in the case of bone metastases Poor results are generally obtained in patients with lung liver node or cerebral metastases

(2) Androgens can be used if relapse occurs leading to a further remission in the dependent types Dosage of at least 200 milligrams testosterone propionate weekly for at least 8 weeks leads to control of metastatic bone pain in about 50 per cent of cases and control of soft tissue growth in about 35 per cent of cases (*Stoll 1950*) for an average of 12 months

(3) If the tumour is non hormone dependent as shown by there being no relief from (1) or (2) cortisone 200–400 milligrams daily is tried This may lead to temporary relief of symptoms for about 3 months only but it can also be used to select cases for adrenalectomy

(4) Adrenalectomy may lead to a further remission in 40–50 per cent for an average of 9 months usually in cases responding to cortisone If there has been no relief from oophorectomy there is *unlikely* to be relief from adrenalectomy and this also gives an indication for selection of cases

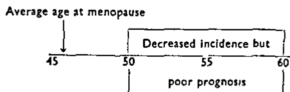


FIG 10

If we assume that the average age at the menopause is 46 years the poorest prognosis would appear to occur from 3 to 13 years after the menopause At the cessation of the menses it takes several years for oestrogenic hormones to disappear from the blood and urine and endometrial biopsies show continuation of oestrogen function (Novak and Richardson 1941) which may originate partly in the adrenal cortex The gradual disappearance of oestrogenic hormones from the circulation after the menopause appears to influence the growth of carcinoma of the breast

Role of pituitary secretion —Reciprocal inhibition between the gonadal and hypophyseal secretion is accepted Meyer in 1932 noted that large doses of oestrogens lead to decrease in the gonadotropin content of the pituitary in rats although Lane in 1934 observed that small doses or short term administration stimulate the release of gonadotropin Since it is well established clinically that oestrogen or androgen administration in high dosage at or after the menopause can decrease the high level of gonadotropin excretion and since these hormones give benefit in postmenopausal cases of breast carcinoma in this age group the tumour is regarded as gonadotropin dependent However a high dosage of oestrogens also inhibits the secretion of somatotropin prolactin and thyrotropin and stimulates the secretion of corticotropin (Tepperman *et al* 1943) It must be remembered that whereas the effect of gonadotropin on the breast must be mediated via the gonads (or extragonadal sources of oestrogen) somatotropin and prolactin can act directly on breast tissue

In premenopausal cases breast carcinoma is thought to be oestrogen dependent On this basis castration by depressing the production of oestrogens should lead to clinical improvement and the administration of oestrogens might be expected to lead to clinical activation of the neoplasm This has been found to be correct in a proportion of cases as will be seen later

Oestrogens —Many authorities have stressed the danger of the recent increase in the therapeutic use of oestrogens Several cases of carcinoma of the breast closely following intensive oestrogen therapy have been reported in the literature since that of Allaben and Owen in 1939 It was suggested by Auchinloss and Haagensen in 1940 that in such cases oestrogens act through the pituitary in causing breast malignancy The incrimination of oestrogens is by no means absolute but publication of the cases has led to their less indiscriminate use Occasional association of breast carcinoma with a high oestrogen concentration from granulosa cell carcinoma has also been noted

Relationship to pregnancy —The higher incidence of carcinoma of the breast in nulliparae is well established (Lane Claypon 1926) Such women are probably not exposed to the fluctuations in hormonal concentrations to which the parous are liable and such hormonal variations may be of protective value It has also been noted (Olch 1937) that menopause delayed beyond the age of 50 years is

oestrogens or androgens may possibly be due to the adrenal cortex altering its secretion to support the growth of the neoplasm

Unfortunately the basic assumption that mammary carcinoma is oestrogen dependent is unable to explain satisfactorily the regression under oestrogen therapy seen in postmenopausal cases and Nathanson (1952) showed that massive doses of stilboestrol could induce a clinical remission even in premenopausal women In addition there are clinical observations that a change from androgen to oestrogen administration or even sudden cessation of androgen administration may lead to further tumour regression in a carcinoma showing reactivation and that androgen may accelerate the growth in some cases (Myers *et al* 1955) These facts cannot be clearly explained by the suggested oestrogen dependence and the only satisfying explanation is to our mind that once established mammary carcinoma is *pituitary dependent* Thus hormone therapy whether by oestrogens or androgens and whatever the age group is dependent on suppression of specific pituitary hormones—probably prolactin and gonadotropin but possibly also adrenotropin or somatotropin Such a mode of control is shown graphically in Fig 11 Inhibition of oestrogens is probably only of secondary importance in the control of breast cancer

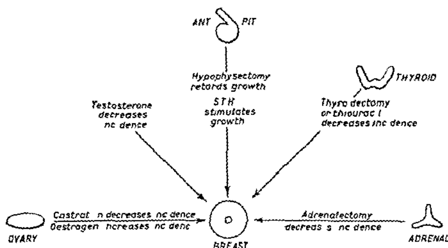


FIG 11 —Hormone control in mammary cancer

Pituitary dependent nature of breast carcinoma

The temporary nature of such hormone control is due to the pituitary eventually compensating for the swing of hormonal balance and control of the pituitary factor is therefore the key to the problem of hormonal control of breast carcinoma Evidence in favour of the pituitary dependent nature of breast carcinoma is as follows

(1) Both androgen and oestrogen administration inhibit pituitary secretion whether in young or old patients Androgens can in any case be partly converted to oestrogens in the body

(2) After hypophysectomy for breast cancer the administration of somatotropin has been shown to cause increase in osteolysis suggesting an important role for

(5) Hypophysectomy may lead to a further remission if relapse after adrenalectomy occurs and many authorities now advise this operation in preference to adrenalectomy Improvement in anaemia with cessation of osteolysis and remission of symptoms may last for 6–12 months or more

Postmenopausal cases

(1) Oophorectomy alone rarely leads to remission but if oestrogens persist in the urine or a vaginal smear shows cornification after the menopause operation is worth while

(2) Oestrogens lead to a remission in 20–30 per cent of cases with bone metastases and in 50–60 per cent of cases with soft tissue metastases for an average of 9 months even if lung metastases are present (Stoll and Ellis 1953) Patients should be at least 3 years postmenopausal otherwise oestrogens are dangerous and may lead to activation of the disease

(3) Androgens usually give better relief than oestrogens in the presence of bone metastases about 60–70 per cent of cases being relieved for up to 12 months

(4) Cortisone administration is followed in responding cases by adrenalectomy

(5) Combined oophorectomy and adrenalectomy leads to relief in 50 per cent of cases the better results being obtained in the presence of bone metastases

Rationale of hormone therapy in breast cancer

Little attempt has been made in the literature to correlate all observed experimental and clinical facts On the basis that the disease is oestrogenic in origin it has been suggested that the established methods of hormone control may act as follows in the hormone dependent cases

(1) Removal of the source of the maintaining hormone for example by castration or by adrenalectomy

(2) Inhibition of maintaining hormone for instance androgens may inhibit oestrogens at the target site in breast carcinoma in premenopausal cases (just as oestrogens may inhibit androgens in prostatic carcinoma)

(3) Inhibition of the controller for example oestrogens or androgens may antagonize the anterior pituitary hormones in postmenopausal cases of breast carcinoma On a similar basis is the use of hypophysectomy in later cases

Huggins and Dao (1954) suggested that carcinoma of the breast is either *ovary dependent* or *adrenal dependent* Oestrogens from the ovary and similar compounds from the adrenals may sustain breast carcinoma Since one cannot determine the type beforehand both oophorectomy and adrenalectomy are carried out simultaneously although prolonged benefit from oophorectomy alone in some cases suggests ovary dependence The benefit derived from oestrogen or androgen administration is considered on this basis to be due to depression of gonadotropic secretion

Pearson *et al* (1954) suggested that carcinoma of the breast is either *oestrogen dependent* or *non oestrogen dependent* In the former reactivation after oophorectomy may be due to the adrenal cortex taking over oestrogen secretion

West *et al* (1952) stress Dobriner s findings that abnormal steroids originating in the adrenal are found in the urine of breast cancer cases They suggest that hormone resistance developing after castration or after the administration of

complete pain relief in metastases was maintained for up to 5 years Oestrogen therapy as reported by Kennedy and Nathanson in 1953 does not appear to be as effective Prophylactic orchidectomy has been suggested following mastectomy in male breast cancer but is not recommended Later in the disease adrenalectomy and hypophysectomy are as useful as in female mammary carcinoma

PROSTATIC CARCINOMA

Endocrine aetiology of prostatic carcinoma

It is more than 150 years since John Hunter noted atrophy of the prostate following castration but the observation appears to have been forgotten until White in 1893 suggested orchidectomy in the treatment of benign hypertrophy of the prostate In 1940 Huggins and Stevens suggested castration in the treatment of prostatic carcinoma and in 1941 Huggins and Hodges reported the benefits of oestrogen therapy in this disease The prostate is normally acted upon by oestrogens and androgens which originate in the patient s testis or adrenal cortex Huggins noted that the administration of oestrogens (or the operation of orchidectomy) depressed the prostatic secretion while androgens stimulated it and these same hormones were noted to have a similar depressive or stimulating effect on prostatic cancer

Experimental observations

It can be shown in almost all animals that oestrogen administration in excess of the normal supply causes atrophy of the prostatic epithelium followed by metaplasia to squamous epithelium In addition there is a concomitant increase in the fibromuscular stroma It is interesting to note that smaller doses of oestrogens cause more rapid changes in the prostate than do larger doses (Chamorro 1950) Benign hypertrophy of the prostate however can rarely be induced by oestrogens except in dogs and there is only one case in the literature by Burrows and Kennaway where oestrogen therapy in an animal led to metaplasia of the prostatic epithelium resembling carcinoma histologically

On the other hand it has been noted that successful grafting of prostatic adenocarcinoma requires an adequate androgen level Horning in 1949 noted tumour regression in such a graft when it was transferred to castrated mice although with repeated transplantation the tumour becomes more and more independent of hormonal control (This is unlike mammary cancer where in the hereditary adenocarcinoma of mice all three factors—heredity oestrogens and the milk factor—are essential for its spontaneous development Once established however the tumour can be easily transplanted and is independent of hormonal control)

The therapeutic effect of oestrogens or of castration in prostatic carcinoma is thought to depend on the mediation of the pituitary gland Scott (1953) noted that preliminary hypophysectomy decreases the prostatic response to androgens but the administration of prolactin increased the sensitivity and is presumably favourable to prostatic growth This observation should be considered in relation to the fact that oestrogens can inhibit the release of prolactin from the pituitary

Clinical observations

It is well known that benign hypertrophy of the prostate in men occurs mainly

this hormone (Pearson *et al* 1954) There is however no experimental evidence that growth hormone secretion can be suppressed by hormone therapy other than hypophysectomy and growth hormone is probably only a co factor in carcinogenesis

(3) Clinical regression of growth after adrenalectomy often occurs without significant changes in the output of gonadotropin or oestrogens although these changes are often seen (Scowen and Hadfield 1955) The clinical response of breast carcinoma to administration of corticotropin in some cases (West *et al* 1952 Segaloff *et al* 1954a) cannot be explained on the oestrogen dependent basis

(4) A much higher proportion of relief is achieved by complete hypophysectomy than by adrenalectomy possibly due to removal of prolactin or somatropin Hypophysectomy is just as effective whether previous adrenalectomy and oophorectomy has or has not been carried out (Luft and Olivecrona 1955)

(5) As has been seen in the previous section breast carcinoma cannot be induced by oestrogens in the absence of the hypophysis It has been suggested that the action of oestrogens in mammary growth stimulation is merely one of increasing the vascularity of the mammary stroma so that access of other hormones such as prolactin is facilitated (Mixner and Turner 1942) and this may apply also to carcinogenesis

(6) Muhlbock (1954) was able to induce mammary carcinoma in mice by implantation of the anterior pituitary gland without the administration of extraneous oestrogens Smith *et al* (1954) increased the growth of mammary carcinoma in mice by administration of growth hormone

There may in addition be peripheral effects from both oestrogens and androgens Thus androgens have a controlling influence on calcium metabolism and this may account for its better control of bone metastases Conversely regression of soft tissue metastases with concomitant progression of bone metastases under oestrogen therapy suggests that the local tissue reaction may be influenced It is established that oestrogens tend to cause proliferation of fibroblasts with tendency to sclerosis and this may decrease the blood supply to malignant cells (Maximow and Bloom 1952)

Mammary carcinoma in the male

The incidence of breast cancer in males is approximately 1–2 per cent of that in females The low incidence may result from the fact that in males it is a vestigial structure and therefore not subject to the cyclic hormonal changes of the female There is no evidence that gynaecomastia is precancerous and heredity appears to play little part in the incidence of male breast carcinoma Lacassagne showed mammary carcinoma to develop in male mice exposed to oestrogenic stimulation and clinically there have been several cases of mammary carcinoma developing in males following prolonged stilboestrol therapy for prostatic cancer since the first report of Abramson and Warshawski in 1948 It is possible that spontaneous breast carcinoma in males is due to the effect of oestrogen over secretion from the Sertoli cells of the testis or from the adrenal cortex A high oestrogen level in the blood may also follow the presence of liver disease (such as that following vitamin B complex deficiency) which interferes with inactivation of oestrogens

In the case of advanced mammary carcinoma in the male Farrow and Adair (1942) reported beneficial results following orchidectomy and in some cases

and hypophysectomy removes the controlling hormone which may activate extra gonadal sources of androgen

Androgen control

The action of oestrogens in this disease has been explained as (1) depression of the gonadotropic secretion of the pituitary and (2) antagonism to peripheral androgens (Murray 1937) at the target site leading to a local effect on the prostatic tissue

With regard to the latter suggestion it has been postulated that oestrogens may have a specific depressant action on the growth of carcinoma cells This supposition is hard to reconcile with the success of surgical castration and adrenalectomy Nevertheless it is possible that oestrogens lead to an increase in the fibrous tissue of the prostate following stimulation of the reticulo endothelial system (Maximow and Bloom 1948) a similar effect may be achieved by orchidectomy

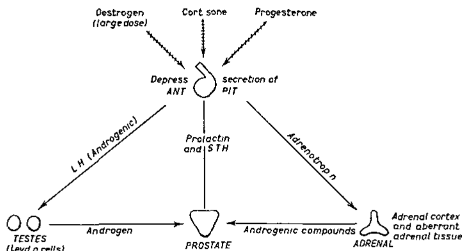


FIG 12 —Hormone control in prostatic cancer

After castration for prostatic carcinoma there is a fall in the urinary 17 keto steroids but when relapse occurs there is a sudden rise in the level according to Huggins presumably due to hypersecretion of androgens by the adrenals After adrenalectomy again there is usually a rapid fall in the 17 ketosteroids There is an *increase* in the excretion of pituitary gonadotropin in the urine both after castration and after adrenalectomy suggesting pituitary release from the inhibition of the gonadal or cortical hormone On the other hand following oestrogen therapy there is a *decrease* in the excretion of pituitary gonadotropin in the urine

These observations make the explanation of androgen control difficult In addition later work showed that prostatic carcinoma could be accelerated by the administration of growth hormone (Kennedy 1956) and clinically controlled by testosterone administration (Brendler *et al* 1950) by progesterone administration (Trunnel *et al* 1951) and by cortisone administration (Hayward 1953) Some recent experimental observations may help to unify all these apparently conflicting

in the median lobe of the gland It has been suggested on experimental grounds that this portion of the gland is the one sensitive to oestrogen and androgen influence and therefore that benign enlargement of the gland in man is due to relative excess of one hormone On the other hand it must be noted that prostatic carcinoma arises in the posterior lobe in the majority of cases Teilum (1950) has demonstrated the relative predominance of lipoids in the oestrogen producing Sertoli cells of the testis in men over the age of 60 years and decrease in lipoids in the androgen producing Leydig cells It is therefore suggested that oestrogen over production may be associated with the development of prostatic tumours Excess oestrogens have however never been demonstrated in the urine of patients with benign or malignant prostatic enlargement It is probable therefore that prostatic neoplasia is caused by a shift in the oestrogen–androgen ratio rather than by actual excess of either hormone bearing in mind that prostatic carcinoma may reflect an endocrinological status existing 10–20 years before the clinical onset of the disease

To summarize oestrogens when relatively in excess of androgens may cause epithelial hyperplasia (which may possibly progress to cancer of the prostate) or may cause hyperplasia of the fibrous stroma (which may progress to benign prostatic enlargement) Both conditions may co exist just as oestrogen induced uterine fibromyoma and endometrial carcinoma may co exist

Rationale of hormone therapy of prostatic cancer

Whatever the hormonal factors leading to its development the continued growth of prostatic cancer once established depends on the presence of androgens Their withdrawal by castration or antagonization by oestrogens causes regression of growth After a certain time however prostatic cancer develops autonomy and no longer responds clinically to hormone therapy Although clinically some cases of prostatic cancer never show hormone sensitivity from the beginning it is possible that there is a phase in the life history of every prostatic cancer (and also of every breast cancer) when it is hormone dependent although this phase may be only transitory

Castration or oestrogen administration lead to atrophy of malignant prostatic tissue with diminution in the blood supply Histological changes include dis appearance of mitotic figures and vacuolation of the cytoplasm According to Moore (1947) irradiation of the testes of patients with prostatic cancer also leads to atrophy of the tubules and interstitial cells decrease in the urinary androgens and relief of clinical symptoms although not as striking as that following orchidec tomy Later work has shown that when the tumour escapes from the control of oestrogens or of castration it can be favourably influenced by adrenalectomy or by hypophysectomy

To assess the benefits of androgen control therapy the results of Staubitz *et al* (1954) are quoted They noted 63 8 per cent of 5 year survivals for treated prostatic cancer without metastases and 45 5 per cent for those with metastases compared with 10 8 per cent for untreated cases

Huggins classifies prostatic carcinoma as either androgen *dependent* or androgen *independent* Based on the conception of the tumour being stimulated by a high androgen level he seeks to eliminate or antagonize all sources of androgen in the body Castration removes the main source adrenalectomy removes a second

steroids from the reticular zone of the adrenal cortex Hyperplasia of the adrenal cortex is known to occur after orchidectomy and after oestrogen therapy (Shipley and Meyer) and this may explain why approximately 50 per cent of relapses in these cases are benefited by adrenalectomy

The acid phosphatase secretion of the tumour and its metastases is to a certain extent an indicator of the activity of the malignant cells Nevertheless the control of oestrogen dosage levels by acid phosphatase levels is not generally considered reliable The serum acid phosphatase level falls in the majority of cases following castration or oestrogen therapy but not so commonly after adrenalectomy

In comparing the effect of orchidectomy and oestrogens Nesbit and Baum (1950) in an analysis of 1 818 cases noted 53 4 per cent 3 year survivals for orchidectomy 49 7 per cent 3 year survivals for oestrogen therapy and 66 per cent 3 year survivals for combined therapy

RECENT ADVANCES IN HORMONE CONTROL

Cortisone therapy

Sprague *et al* (1950) suggested that as cortisone administration leads to depression of corticotropin secretion and atrophy of the adrenal cortex effects similar to those of adrenalectomy might be achieved by its use in hormone sensitive cancers This has been clinically confirmed and cortisone has the advantage that it stimulates the appetite and seems to improve haemopoiesis Valk and Owen (1954) reported clinical benefit in prostatic carcinoma from cortisone administration with a rise in the urinary gonadotropin level and a reduction in the serum acid phosphatase level in the majority of cases They noted better results in the case of a well differentiated prostatic carcinoma The dose of cortisone suggested is from 200 to 400 milligrams daily for 2 weeks

Subjective benefit from cortisone including relief of bone pain can be expected in the majority of cases although objective signs of remission or recalcification of bone metastases are rare Long continued administration may even lead to osteo porosis It is used for relapse of symptoms following oestrogen administration or orchidectomy although relief of symptoms generally lasts for a few months only and is not as complete as that following adrenalectomy Nevertheless it is useful for patients who are seriously ill and cannot stand a severe operative pro cedure Pearson *et al* (1954) in discussing the treatment of advanced mammary carcinoma note that cortisone may be useful also for non oestrogen dependent tumours where adrenalectomy has failed Corticotropin administration may also be beneficial thus West *et al* (1952) showed that corticotropin therapy given in the presence of bone metastases from mammary carcinoma may lead to relief of symptoms and Segaloff (1954) reported that corticotropin or cortisone administra tion led to clearing of lung infiltration in 2 cases

It has been suggested that as cortisone can suppress adrenal secretion as effectively as adrenalectomy a trial of cortisone therapy is indicated before adrenalectomy is considered Pearson s observation of the fall in urinary calcium excretion after hormone therapy in the presence of osseous metastases has been expanded into a test for hormone dependency by Emerson and Jessiman (1956) (*see* below)

reports Reifenstein *et al* (1945) demonstrated a *decrease* in the urinary 17 keto steroids following administration of methyltestosterone Based upon experimental evidence they suggested that this decrease resulted from reduction of luteinizing hormone (LH) output and that androgen production both by the adrenal and testis was under the influence of LH Based upon this hypothesis Fig 12 represents the possible interaction of endocrine controllers in prostatic carcinoma

It should be noted that the presence of prolactin is known to increase the prostatic response to testosterone (Scott 1953) and Fiori *et al* have shown that relatively low doses of prolactin stimulate the secretion of testosterone in rats Scott showed that oestrogens inhibit the release of prolactin from the pituitary and the fact that prolactin is identical with luteotropin in the rat was suggested by Evans *et al* (1941) and confirmed by many reports since The evidence in the human is not so clear

If one assumes that androgen secretion and therefore relapse of symptoms in prostatic carcinoma is controlled by the LH of the hypophysis the following rationalizations are possible

(1) Relapse after long continued oestrogen therapy is due to eventual escape of LH secretion and increased androgen secretion will follow

(2) Reactivation after castration is due to adrenotropin stimulating increased androgen secretion by the adrenal and relapse after adrenalectomy is due to the presence of aberrant accessory adrenal tissue

(3) Response to cortisone androgen or progesterone is due to suppression of LH Cortisone may also have a direct effect on the malignant cells apart from possible suppression of prolactin or of somatotropin

Here again as in breast carcinoma the pituitary secretion holds the key to control of the growth as the presence of prolactin appears to favour the prostatic response to androgen and the luteinizing hormone of the pituitary may control androgen secretion from whatever source Pituitary somatotropin may exert a separate and direct influence on the prostate

Oestrogen therapy and castration

Following oestrogen administration in prostatic cancer there is often spectacular relief of symptoms pain from bone metastases sometimes being relieved after 24 hours The daily dosage of stilboestrol recommended varies from 1 to 100 milli grams and in 1953 Ferguson considered the higher dose level more efficacious when the tumour becomes resistant Ethinyloestradiol given at $\frac{1}{10}$–$\frac{1}{20}$ of the dose of stil boestrol causes less gynaecomastia and nausea (Stoll 1955) The use of TACE (tri *p* anisylchloroethylene) has been recommended as having an oestrogenic effect without any effect on the adrenals or the pituitary Its proponents claim that it avoids gynaecomastia and is longer acting than stilboestrol

Only about one third of cases fail to respond clinically to oestrogen administra tion these cases having mainly anaplastic carcinomas Spread of the neoplasm can be inhibited by oestrogens for up to 10 years in about 20 per cent of cases although relapse occurs in the majority after 2–3 years

Ferguson and Franks in 1953 showed regression of prostatic cancer on oestrogens with associated histological evidence of cell destruction It is possible that reactivation of neoplasm is partly due to the development of independence from androgens of the cancer cell but it may also result from increased secretion of

of radioactive gold seeds into the pituitary via the nose and sphenoidal sinus In 50 cases there was no mortality or complications resulting

Surgical hypophysectomy for hormone dependent cancer was introduced by Perrault in 1952 in the treatment of relapses after adrenalectomy Most investigators report that hypophysectomy is a less severe procedure with less post-operative discomfort and easier hormone maintenance than after adrenalectomy The operation not only suppresses gonadal and adrenal function with atrophy of accessory adrenal tissue but also removes the controlling influence of prolactin and somatotropin There is an immediate fall in the gonadotropin and prolactin levels in the urine and clinical thyroid deficiency with a fall in serum protein bound iodine Cortisone 50 milligrams and sometimes thyroid 120 milligrams daily are given as replacement therapy Clinical manifestations of the disease regress in a high proportion of cases but improvement has been short lived in the majority Hypophysectomy is followed by a remission in about half the cases receiving a remission from previous adrenalectomy

Pearson et al (1954) reported improvement in anaemia and cessation of osteolysis following hypophysectomy in advanced breast cancer with bone metastases These authors make the interesting observation that in one case when beef pituitary growth hormone was administered after the operation there was a temporary increase in osteolysis suggesting that pituitary somatotropin is an important factor Luft and Olivecrona (1955) reported improvement in 17 out of 30 cases of breast cancer following hypophysectomy with *intact* ovary and adrenal gland Andersson (1956) reported that of 20 patients with advanced breast carcinoma treated by hypophysectomy pain was completely relieved in 9 and the growth of metastases retarded It is suggested that failure of relief from hypophysectomy is due to incompleteness of the operation and only rarely to pituitary independence (Hadfield 1956) as experiments show that pituitary function is re-established quickly if even a tiny portion of the gland is left after operation If the pituitary dependence of mammary and prostatic cancer is accepted then no benefit from oestrogen or androgen therapy would be expected after *complete* hypophysectomy and this is the case in the few observations noted so far Theoretically too hypophysectomy should yield a summation of the benefits of castration and adrenalectomy as the operation is followed by virtually complete atrophy of the gonads and adrenal cortical tissue but it is too early to establish this point clinically

Selective medical hypophysectomy has been suggested as a possibility in the case of breast carcinoma Although both oestrogens and androgens have the power to inhibit anterior pituitary secretion both these hormones may have unpleasant side-effects—nausea vomiting and uterine bleeding in the case of the former and virilization and fluid retention in the case of the latter Therefore a search is proceeding for a material which will induce pituitary inhibition without the undesirable oestrogenic effects so that it can be used also in the younger age group without danger of activating breast carcinoma In a preliminary report on the use of parahydroxypropiophenone for this purpose (Stoll 1956) the results have been encouraging

FUTURE PLANNING OF HORMONE CONTROL

A planned investigation of hormone control in hormone sensitive tumours requires knowledge of the endocrine and biochemical status of the patients including

Adrenalectomy

The introduction of cortisone replacement therapy enabled Huggins and Bergenstal in 1951 to report the results of bilateral adrenalectomy in the treatment of advanced breast and prostatic cancer In cases of carcinoma of the prostate adrenalectomy leads in the majority to dramatic relief of bone pains but in spite of this radiographically the skeletal metastases usually continue to spread The operation is followed by a sharp fall in the 17 ketosteroid level and increase in the urinary gonadotropin level but not always by a fall in the acid phosphatase level It has been suggested that pain relief following adrenalectomy may be a result of altering the entire endocrine response to stress and painful stimuli (Fischer Williams 1956) as the pain reaction depends physiologically on an intact sympatho adrenal system It is of interest that in some cases of advanced prostatic or breast cancer showing temporary spontaneous regression necropsy examination has shown metastatic replacement of the adrenal glands

It is generally advised that adrenalectomy be performed in breast carcinoma after failure of response to previous castration androgen or oestrogen therapy Huggins and Dao (1953) stated that previous failure of these measures does not preclude the likelihood of response to adrenalectomy and this view is supported by Taylor *et al* (1953) On the other hand from their experience West *et al* (1952) and Pearson *et al* (1954) believe that cases not responding to oophorectomy or testosterone administration do not respond to adrenalectomy

The operation is generally carried out concurrently with oophorectomy (if this has not already been done) and even in the best hands is associated with a mortality of about 5 per cent (Huggins and Dao 1954) It is followed by subjective relief of symptoms in about 50 per cent of cases but objective improvement in skin gland visceral or bone metastases is seen in only about half of these cases Relief of symptoms occurs from 1 to 3 weeks after operation the results are often dramatic Relief may last up to 3 years but 12–18 months is more usual Whereas Cade (1955) and Huggins and Dao (1953) observed that the results are better in well differentiated mammary adenocarcinoma Galante *et al* (1954) found that the results are as good in the case of anaplastic carcinoma In addition Huggins found that the results are better in premenopausal patients and regards hot flushes after adrenalectomy as a good prognostic sign indicating over action of the pituitary In this respect Huggins and Dao (1954) noted that a high titre of oestrogens in the urine before operation and a high level of gonadotropin following adrenalectomy indicated a good prognosis

The presence of accessory adrenal tissue which may be activated after adrenalectomy is suggested in the cases of Pearson *et al* (1954) some of whom required no cortisone maintenance therapy after adrenalectomy

Hypophysectomy

In 1945 Herbst and Sauer suggested irradiation of the pituitary gland in the treatment of cancer but Kelly in 1951 reported that pituitary function cannot be measurably reduced clinically or biologically even by high doses of radio therapy to the hypophysis Nevertheless implantation of radon seeds into the pituitary and the injection of colloidal radioactive phosphorus (Rothenberg *et al* 1955) have been tried in some cases and relief of pain from bone metastases in advanced carcinoma has been claimed Greening (1956) reported on the insertion

of radioactive gold seeds into the pituitary via the nose and sphenoidal sinus In 50 cases there was no mortality or complications resulting

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FUTURE PLANNING OF HORMONE CONTROL

A planned investigation of hormone control in hormone sensitive tumours requires knowledge of the endocrine and biochemical status of the patients including

Oestrogenic androgenic status

The oestrogenic-androgenic status of patients with advanced mammary or prostatic cancer should be determined as a preliminary to institution of hormone therapy. Both bioassay and chemical analysis of urine should be made and correlated both with the rate of cancer growth and also with the clinical effect of different types of hormone therapy such as steroid administration castration adrenalectomy and hypophysectomy

In 1941 Pincus and Graubard showed higher urinary oestrogen levels in post menopausal patients with breast cancer than in normal patients of the same age group. Smith and Emerson (1954) confirmed this finding and noted that cortisone administration or surgical castration lowered these levels to normal. Struthers (1956) on the other hand by assay of vaginal cornification suggested that there is no greater oestrogen production in patients with breast carcinoma after the menopause

Estimation of urinary excretion of pituitary hormones

Estimation of urinary gonadotropin prolactin or somatotropin excretion should be carried out and may be related to the hormone dependency of breast cancer. In this respect Hadfield (1956) using a new method of urine extraction has investigated a mammotropic factor (present in human premenopausal urine and in the urine of 50 per cent of postmenopausal women) which has no oestrogenic activity and disappears after hypophysectomy. It is demonstrable by its stimulating effect on the mammary duct system in mice and its presence according to Hadfield indicates a pituitary dependent tumour. Scowen and Hadfield (1955) found that although castration may influence tumour regression associated with fall in the oestrogen or gonadotropin levels there is often no change in oestrogen or gonadotropin levels in the urine following adrenalectomy. Therefore some other pituitary hormone such as prolactin may be of greater importance. Segaloff *et al* (1954b) showed that a good prognosis from equinelene administration in mammary cancer was associated with a fall in urinary prolactin excretion and a bad prognosis with a rise

Andersson (1956) in his report on 20 cases of hypophysectomy for breast cancer suggested that somatotropin is essential for the growth of metastases. Activation of osteolysis by the administration of somatotropin after hypophysectomy (Pearson *et al* 1954) merits further investigation

Calcium metabolism

Spontaneous hypercalcaemia is often noted in the presence of bone metastases in these tumours and may be increased by androgen or oestrogen administration. A rise in the serum alkaline phosphatase and a fall in the serum phosphorus has been noted with successful hormone therapy (Pincus 1956). The report of Emerson and Jessiman (1956) that changes in the urinary calcium excretion can be used to determine the hormone dependency of mammary or prostatic cancer in the presence of bone metastases has given a method of predicting in advance the response to operation such as adrenalectomy

Ketosteroid and ketogenic steroid excretion

Investigation of ketosteroid and ketogenic steroid excretion before and after

treatment may be helpful in assessing the adrenocortical changes in hormone therapy

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CHAPTER 18

ENDOCRINE TREATMENT OF GYNAECOLOGICAL DISORDERS

P M F Bishop

INTRODUCTION

For some years now there has been no major advance in the endocrine treatment of gynaecological disorders and it seems unlikely that there will be until the mechan ism of ovulation and its control in the human being is better understood and until such physiological knowledge can be translated into practical therapeutics Nevertheless there are some solid achievements in this field Three examples are the prevention of dysmenorrhoea by suppressing ovulation the arrest of metro-pathic bleeding and the postponement of future episodes by inducing progesterone withdrawal bleedings and the relief of menopausal symptoms Though the clinician may have been barren of new ideas the pharmaceutical chemist has in contrast been most active Since 1950 a considerable number of new oestrogenic compounds have been introduced

CHOICE OF OESTROGENS

Synthetic compounds with minimal side-effects

Such a compound has recently been introduced and reported upon by Sturnick and Gargill (1952) It is methoxy naphthyl dimethylpentanoic acid and is referred to as vallestril It is a derivative of allenolyic acid one of the group of carb-oxylic acid compounds The dose required about 4 5 milligrams daily for control of menopausal symptoms is greater than that of other synthetic oestrogens but the compound is claimed to be singularly free of side effects and no patient in the authors series has experienced oestrogen withdrawal bleeding on discontinuing treatment One patient complained of nausea when taking 7 5 milligrams daily

Potent natural oestrogens

Attention was first directed to urinary extracts by Marrian (1930) who had already isolated oestrol from this source *Oestriol glycuronide* was one of the earliest oestrogens to be given a clinical trial and other water soluble conjugated oestrogens were sought in the urine of pregnant mares One of these *sodium oestrone sulphate* was prepared synthetically by Butenandt and Hofstetter (1939) A mixture of these *equine conjugated oestrogens* (Premarin) was put on the market in Canada and eventually distributed in Great Britain However the demand was so great in the North American continent that supplies became unavailable in the United Kingdom the preparation has only recently been reintroduced here It is a highly potent oral natural oestrogen and was found by the present author and his colleagues to be half as active as stilboestrol

Further studies have been made with compounds similar to equine conjugated oestrogens Synthetic sodium oestrone sulphate and piperazine oestrone sulphate have been studied in 58 menopausal women by Perloff (1951) who found them highly effective in average daily doses of about 2 milligrams with low incidence of undesirable side-effects Perloff considered that sodium oestrone sulphate has an activity similar to equine conjugated oestrogen and suggested that it is the active agent of the conjugated compound Kupperman *et al* (1953) however found equine conjugated oestrogens to be considerably more potent than sodium or piperazine oestrone sulphate Reich *et al* (1952) studied the effect of piperazine oestrone sulphate on 100 menopausal women and found that daily doses of from 0 75 to 6 75 milligrams were required depending on the severity of the symptoms In 2 patients profuse withdrawal bleeding occurred despite the fact that intervals were allowed to elapse between successive courses No significant advantage was seen when piperazine oestrone sulphate was combined with an androgen Some patients are offended by the odour of the naturally occurring equine conjugated oestrogens and piperazine oestrone sulphate is said to be odourless An advantage of these water soluble oestrogens is that they are most effectively absorbed when administered topically for instance as a lotion or cream applied to the skin in cases of acne Furthermore they can be injected intravenously which considerably accelerates their haemostatic effect in cases of alarming bleeding in metropathia haemorrhagica

Long acting oestrogens

For some time there has been a vogue especially in the United States of America for long acting steroid preparations Pellet implantation has gone out of fashion though it would seem to the author to be the most economical and efficient form of long-continued substitution therapy He would agree that there are few if any indications for it in gynaecology Protracted administration of oestrogens will usually induce metropathic bleeding and prolonged androgen therapy hirsutism or voice changes Furthermore in the case of oestrogens where there are compounds which are effective when given by mouth there seems to be no particular advantage in submitting the patient to intramuscular injection merely to achieve a prolonged effect For this an appropriate ester must be found and a considerable number of such esters are on the market Another method is to inject a crystalline aqueous suspension of the hormone In Great Britain at the present time oestradiol cyclopentylpropionate oestradiol valerianate and oestradiol undecylate are available for clinical use

Robinson (1953) has reported on the use of oestradiol cyclopentylpropionate on 253 menopausal patients followed for more than a year It was usually administered in combination with testosterone cyclopentylpropionate Single injections of the oestrogen in doses of 1–3 milligrams brought relief for 3–6 weeks and were not accompanied by any side-effects or withdrawal or break through bleedings when given in combination with the androgen The majority of the women were receiving 2 milligrams of oestradiol cyclopentylpropionate and 100 milligrams of testosterone cyclopentylpropionate once every 4 weeks This caused hirsutism in some cases Schwartz and Soule (1955) studied the effects on the vaginal smear the menopausal symptoms and the duration of onset of oestrogen withdrawal bleeding after a single injection From this they calculated that 25 milligrams of oestradiol cyclopentyl propionate remained effective for from 30 to 36 days and produced a withdrawal

bleeding and tenderness and swelling of the breasts in about half the 21 cases treated A dose of 5 milligrams remained effective for 24 days but produced no symptomatic relief and caused withdrawal bleeding in 1 out of 9 cases In comparison the duration of response to 5 milligrams of oestradiol benzoate or dipropionate was 17 days and only a weak response was achieved Studying the effect on vaginal smears in the menopausal patient Wied (1954) claimed that 10 milligrams oestradiol valerianate remained effective for about 3 weeks

A fat soluble oestrogen

The synthetic compound tri *p* anisylchloroethylene (chlorotrianisene or TACE) is a pro oestrogen which is stored in the body fat and converted by the liver into a potent oestrogen Because of its storage in the fat depots it is claimed that it is released gradually and constantly to the tissues thus providing a smooth and prolonged effect and that its release is in gradually diminishing concentrations so that it imitates the waning of ovarian function at the climacteric and it lessens the tendency to oestrogen withdrawal bleeding

Rothchild and Keys (1952) found that 4 daily doses of 6–12 milligrams of TACE produced complete cornification of the vaginal mucosa but that these doses were no more lasting in effect than when other oral oestrogens such as ethinyloestradiol were given Benson and Garetz (1953) who described this compound as an oral implant found that doses of less than 24 milligrams daily do not produce a prolonged effect In the lower doses they employed withdrawal bleeding was experienced in some cases Gillam *et al* (1954) studied the effect of TACE on 53 menopausal patients in doses of 24 milligrams daily for 2 weeks followed by 12 milligrams daily for another 2 weeks Hot flushes were relieved in 49 out of 51 cases In 14 cases hot flushes returned within 7–43 days after the end of the course so that a second course was required Side effects were uncommon but in 2 cases heavy withdrawal bleeding requiring curettage was experienced Woodhull (1954) studied the effect of this compound in 87 menopausal cases with hot flushes The usual dose was 12–24 milligrams daily by mouth for 1–2 months and symptomatic relief was obtained in 91 per cent of the cases The duration of effectiveness was variable being longer the less severe the symptoms the average duration of relief was 3 months In 39 per cent of the patients symptoms did not return sufficiently severely to require a further course of treatment during the period of observation which was from 3 to 14 months Out of 71 patients with a natural (as opposed to artificial) menopause there were only 3 instances of withdrawal bleeding Woodhull commented on the smoothness of action of the compound and the patient s sense of well being which was greater than that obtained with other oestrogens The author s experience is that withdrawal or break through bleeding is the rule rather than the exception and in 2 cases has necessitated admission curettage and transfusion

Pituitary stimulant with minimal oestrogenic activity

Werthessen and Gargill (1945) having found that certain ethers of stilboestrol and in particular the monobenzyl ether showed a marked pituitary potentiating capacity in rabbits undertook clinical trials with this compound (Werthessen and Gargill 1952) In 10 menopausal women and 1 case of ovarian agenesis used as controls stilboestrol had previously suppressed symptoms cornified the vaginal mucosa and lowered urinary gonadotrophin levels The monobenzyl ether however in the doses given showed no oestrogenic effect according to these criteria The experimental group consisted of 18 patients with secondary amenorrhoea of

at least a year's duration who were anxious to become pregnant. Several had received stimulating doses of deep x rays to the pituitary, ovaries or both. Each patient was given an injection of 1 milligram thrice weekly for 3 weeks. Nine patients received one course only and failed to menstruate. The remaining 9 menstruated after a second or third course. 5 of these resumed cyclical menstruation and 3 became pregnant. An additional patient treated with the monobenzyl ether in daily doses of 4.5 milligrams for 20 days each month, ovulated after the fourth month and became pregnant a month later. Jungck and Brown (1953) deny the absence of oestrogenic effect and found that 3 milligrams of the monobenzyl ether given by mouth for 10 days produced effects on the vaginal smear of menopausal women similar to those obtained by 1 milligram stilboestrol.

NEW PROGESTOGENIC COMPOUNDS

It was commonly believed that progesterone is effective only when administered by intramuscular injection and indeed the compound 17-α ethinyltestosterone, variously known as anhydrohydroxyprogesterone, pregneninolone, ethinyltestosterone or ethisterone as it is referred to by the British Pharmacopoeia (1948), is commonly employed when an oral preparation is required which possesses progestogenic properties. Greenblatt *et al* (1950), however, have studied the effect of giving progesterone by mouth in doses of 80-100 milligrams daily for 5 days to amenorrhoeic women, some of whom had been previously primed with oestrogen. Withdrawal bleeding occurred following 90 per cent of the courses given, though a total of 400-500 milligrams over a period of 3-5 days was insufficient to induce secretory changes in the endometrium in one case. Greenblatt (1956), however, subsequently pointed out that oral progesterone was only a third to a fifth as effective as ethisterone and that ethisterone was only a fifth as effective as parenteral progesterone. A new orally active compound, 17-α ethinyl 19 nortestosterone (*norethisterone*) has now been introduced and Greenblatt (1956) found that it would produce withdrawal bleeding with as small a dose as 50 milligrams over a period of 5 days and would induce secretory endometrial changes with a total dose of 170 milligrams over a period of 12 days. He estimated that this compound was about twice as active as ethisterone and about half as effective as parenteral progesterone. Ferin (1956) has reported on an even more potent oral—or rather sublingual—progestogen, 17 methyl 19 nortestosterone (17 α methyl 17 β hydroxy 3 keto oestr 4 ene, methyloestrenolone or *methylnortestosterone*). The criterion used was the production of subnuclear vacuolation in the epithelial cells of the endometrial glands, an indication of a massive glycogen load which is present only in a progestational endometrium. Ferin claims that methylnortestosterone is 150 times as active as ethisterone when given by the sublingual route and about 10 times as active as progesterone by injection.

The vogue for depot therapy affected the progestogens as well as other steroids, and aqueous suspensions of microcrystals of progesterone were claimed to be more effective than an intramuscular injection of an oily solution because the depot effect of the crystals led to better utilization as the result of slower absorption over longer periods. Masters *et al* (1952) compared the effect of these two preparations in producing withdrawal bleeding and endometrial changes in menopausal women primed with oestrogen.

and concluded that the oily solution was at least 4 times as effective and produced a more advanced luteal effect on the endometrium

It has been pointed out that one method of prolonging the effectiveness of these steroid hormones is to esterify them Progesterone contains no hydroxyl groups and therefore cannot be esterified 17 hydroxyprogesterone has no progestational activity however when it is esterified with acetic or caproic acid striking progestogenic effects are produced Davis and Wied (1955) compared the results produced by a single injection of 350 milligrams of 17 α hydroxyprogesterone-caproate with those of pure progesterone in oil in 3 castrated women previously primed with oestradiol valerianate The oestrogen was also administered with the progestogen Withdrawal bleeding occurred from 14 to 19 days after administration of the ester and only a week after the pure progesterone and the endometrium was fully secretory when the ester was given and practically non secretory when the pure compound was used

ANDROGENIC PREPARATIONS

The two original androgens in use were testosterone propionate a parenterally administered ester with a moderately prolonged effect (injections are given thrice twice or even once weekly) and methyltestosterone which is orally effective Modern trends have been concerned with the elaboration of long acting androgens and of those without virilizing side effects A number of long acting androgens are now on the market in Great Britain among these are testosterone phenyl propionate testosterone cyclopentylpropionate testosterone oenanthate and testosterone hexahydrobenzoate

According to Lloyd and Fredericks (1951) testosterone cyclopentylpropionate prolongs the duration of effectiveness to 28 days or more Ott *et al* (1952) compared the effectiveness of testosterone cyclopentylpropionate with that of testosterone propionate and found that the former was much more active and with an androgenic effect several times more prolonged

Since it became recognized that androgens were capable of causing nitrogen retention a search has been made for compounds which are predominantly protein anabolic with relatively feeble androgenic properties Methylandrostenediol was synthesized by Ruzicka *et al* (1935) and was found in animal studies to be a relatively feeble androgen though its anabolic properties were established in animals by Gordan *et al* (1950) They stated that it caused weight gain and nitrogen retention in human subjects (Gordan *et al* 1951) Hall (1951) reported on the treatment of 53 women with *methylandrostenediol He found no signs of virilism or hirsutism such as would be* expected to develop in at least a few of the patients if they had been treated with comparable doses of testosterone derivatives (1 patient received a total of 2 800 milligrams in 2 months) Partridge *et al* (1953) performed careful nitrogen potassium and phos phorus balance studies as well as clinical observations on a small group of patients and concluded that a dose of 200 milligrams or more daily by mouth was required to induce significant anabolic effects and that in such doses androgenic manifestations would be the same as those to be expected with comparable doses of testosterone propionate They considered that methylandrostenediol offered no clinical advantages over testosterone preparations Foss (1956) using methylandrostenediol in doses of 100 milligrams daily in the treatment of patients with inoperable carcinoma of the breast found it almost as virilizing as testosterone propionate or methyltestosterone in comparable doses

FUNCTIONAL UTERINE BLEEDING

The therapeutic objects to be achieved in this condition are to stop a prolonged bout of heavy bleeding in cases where ovulation is not taking place and to prevent its recurrence and to modify the excessive bleeding (menorrhagia) which sometimes characterizes ovulatory cycles

Oestrogen "haemostasis"

One method of arresting the bleeding of a haemorrhagic episode is to administer oestrogen intensively over a short period of time This method was introduced by Karnaky (1940) who recommended from 10 to 25 milligrams stilboestrol every 15 minutes until the bleeding stops usually within 4 6 hours The régime has been modified by a number of workers and the author now gives 0 2 milligram ethinyl oestradiol every 2 waking hours until the bleeding is appreciably checked This is usually within 36–48 hours Greenblatt and Barfield (1951) reported on the use of the water soluble equine conjugated oestrogen given intravenously in doses of 20 milligrams (as standardized in terms of sodium oestrone sulphate) every 6–12 hours for 2–4 doses Later (Greenblatt and Barfield 1952) they recommended that the injections should be given every 4–6 hours The withdrawal bleeding which would be expected to follow this treatment was prevented in the majority of cases from occurring by giving equine conjugated oestrogen by mouth in diminishing doses from 5 to 1 25 milligrams daily for the next 25 days Bleeding was arrested or reduced by the intravenous therapy in 84 2 per cent of 57 courses given in cases of functional uterine bleeding Greenblatt and Barfield (1952) also suggested another method which in their hands was even more satisfactory An intramuscular injection of a combination of 1 66 milligrams oestradiol benzoate 25 milligrams testosterone propionate and 25 milligrams progesterone was given daily for 5 days Bleeding was usually arrested within 6–48 hours If the bleeding was very severe the dose was doubled The authors claimed that this regime controlled nearly 90 per cent of all functional bleeding

Progesterone withdrawal bleeding

It seems well established that natural menstrual bleeding results from the withdrawal of the influence of oestrogen and progesterone Provided that the endometrium is primed with oestrogen subsequent administration of progesterone should lead to withdrawal bleeding a few days after it is discontinued Albright (1938) described this procedure as a medical curettage In the author s hands this has proved a most satisfactory method of indefinitely postponing the next metropathic bleeding episode in metropathia haemorrhagica A course of daily injection of progesterone 25 milligrams is given for 4 days once every 28 days In the majority of cases a withdrawal bleeding usually resembling a normal period follows 2 or 3 days after each course The same effect can often be obtained by a course of ethisterone in daily oral doses of 50 milligrams for 10 days

McArthur (1953) stressed the value of examining vaginal smears in selecting the cases which should be expected to respond to this regime So long as a good cornification index is present it can be assumed that the endometrium has been adequately primed by endogenous oestrogen in which case withdrawal bleeding should take place following the administration of progesterone alone If the vaginal smear shows poorly cornified

cells endogenous oestrogen priming may be inadequate in which case the events of the normal ovarian cycle should be imitated by giving oestrogen and following this by administration of oestrogen and progesterone

Holmstrom (1954) advocated adoption of the principle of progesterone withdrawal bleeding in the treatment of anovulatory cycles He advised a single injection of either 25 or 50 milligrams progesterone which he claimed converts a proliferative endometrium into a secretory type with shedding about 4 days later The single injection of pro gesterone is then repeated on either the twenty fourth or the thirtieth day of subsequent cycles until there is evidence that ovulation is occurring spontaneously Few perhaps would agree that the progesterone initiates the serial reaction which results in sub sequent spontaneous ovulatory cycles In fact spontaneous remission of metropathia haemorrhagica in the absence of treatment is a common experience

Cyclical hormone therapy

This consists of administration of small physiological doses of oestrogen for the first 2 or 3 weeks of the cycle followed by progesterone during the third week There is no standard dosage but the objective is to imitate the events of the normal ovarian cycle in cases in which it is believed that the ovary is producing too little oestrogen The author sees little advantage in the preliminary oestrogen unless the endometrium is so atrophic that it is unlikely to shed following pro gesterone administration

Oestrogen therapy to prevent bleeding

This is what is referred to by many American authors as oestrogen haemo stasis though in Great Britain the term is used for the intensive oestrogen therapy already described and designed to stop bleeding (Bishop 1951) Its object is to raise the effective oestrogen level above the bleeding threshold and here it resembles the rationale of the oestrogen haemostasis as practised in Great Britain Its further object is to suppress gonadotrophin release by the pituitary preventing stimulation of the ovary and so producing as Allen (1951) believes a temporary castration effect It is usual to administer the oestrogen in relatively high doses—about 5 milligrams stilboestrol or its equivalent—daily for the first 3 weeks of each cycle a withdrawal bleeding occurring a few days later It is wise to allow a week to elapse between successive courses

Androgen therapy

Huffman (1953) suggests that androgens diminish pituitary activity and thus halt the ovarian and endometrial cycles It is doubtful whether they act in this way in the small doses usually employed and they may have a direct effect on the endometrium or its vessels The author administers oral methyltestosterone in daily doses of 10 milligrams for 2 months allowing at least a month to elapse between successive courses It is often unnecessary to repeat the course for several months Androgen therapy seems to be especially useful in cases of menorrhagia with ovulatory cycles

Swyer (1956) found that he obtained better results by giving a combination of 10 milligrams methyltestosterone or androstanolone and 30 milligrams ethisterone daily for a week before the expected onset of the period until the third day of bleeding than when he gave 10 milligrams methyltestosterone alone The author has found

it difficult to get the correct timing with such a régime because the cycles are often shorter than expected He believes that a more effective diminution of menstrual loss can be obtained by giving androgen continuously for 2 months and that with this plan the loss remains tolerable for a number of subsequent cycles

ANOVULATORY BLEEDING AND THE INADEQUATE ENDOMETRIUM

It is the opinion of those with much experience of treating amenorrhoea and anovulation that it is hardly worth while except *in an attempt* to overcom involuntary infertility Nevertheless papers claiming successful results do occasionally appear but few in which the disappointing results are stressed Individual therapeutic regimes vary considerably

Oestrogen therapy

The commonest method of using oestrogen is to employ just sufficient to induce withdrawal bleeding in the hope that it will release pituitary gonadotrophin as indicated by the studies of Brown *et al* (1953) This may be achieved with about *1 milligram stilboestrol or its equivalent daily for 2 or 3 weeks* It is usual to give about 3 courses and determine whether ovulatory cycles occur subsequently Some authors advocate booster oestrogen therapy to induce or improve ovulation

Paschkis *et al* (1954) mention doses of 0 1–0 25 milligram stilboestrol for 4 days before the expected time of ovulation to release luteinizing hormone and induce ovulation in patients with slow rising basal temperature curves Weir *et al* (1954) gave a gradually increasing dose (for example from 1 25 to 2 5 milligrams equine conjugated oestrogen) from 2 to 5 days ending on the tenth or eleventh day of the cycle to produce a more normal ovulation Another device has been to administer a large dose from 5 to 10 milligrams stilboestrol on about the eighth day of the cycle to suppress and subsequently release gonadotrophin as a rebound effect and in order to induce ovulation

Oestrogen progesterone therapy

This consists in giving oestrogen first and then oestrogen and progesterone to imitate the events of the normal ovarian cycle Siegler (1952) treated 36 patients complaining of infertility and anovulatory cycles with ethinyloestradiol 0 15 milligram daily for 20 days and from 20 to 60 milligrams of oral progesterone from the fourteenth to the twenty fourth day of the cycle with the result that 12 patients ovulated and 2 became pregnant Paschkis *et al* (1954) added for the last 10 days 20–50 milligrams ethisterone to their 21 day stilboestrol regime on the basis that progesterone makes the ovary more responsive to gonadotrophic stimulation Combinations of oestrogen and progesterone are on the market both in oral and parenteral form These are to be given for 3–5 days each month in amenorrhoea to induce withdrawal bleedings or commencing on the twenty second day of an anovulatory or inadequate cycle with the ultimate object of inducing normal ovulatory cycles

Progesterone therapy

The belief is held that progesterone is more effective than oestrogen in stimulating

subsequent ovulatory cycles In amenorrhoea of not more than 6 months standing 25 or 50 milligrams progesterone may be injected daily for 3 days at monthly intervals or from 30 to 50 milligrams ethisterone given daily by mouth for 10 days commencing 2 weeks before the expected onset of the period in anovulatory cycles Kimbrough and Israel (1950) advised injecting 50 milligrams progesterone on the tenth twelfth fourteenth and sixteenth days of the cycle in cases of anovulatory bleeding and repeating the course for 3 successive months

Gonadotrophin therapy

Though most authorities agree that gonadotrophins are disappointing in the treatment of ovulatory failure there are a number of reports in which success has been claimed A course of follicle stimulating hormone is followed by chorionic gonadotrophin in low dosage such as 400–800 i u (international units) of an extract of pregnant mares serum for about 5 injections followed by 500 i u of chorionic gonadotrophin on alternate days for 3–5 injections

Alternatively gonadotrophin therapy can be given in relatively high doses according to the Rydberg technique (Rydberg and Madsen 1948) in which 3 000 i u of pregnant mare serum daily for 5 injections are followed by 1 500 i u on alternate days for 3 injections This may be repeated at monthly intervals About 50 per cent of the women with secondary amenorrhoea in their group became pregnant within 6 months of such treatment

These high doses occasionally induce intense activation of the ovaries which can become considerably enlarged owing to the formation of numerous corpora haemorrhagica From these doses bleeding into the peritoneal cavity may occur producing an acute abdominal emergency If this should happen every effort must be made to preserve as much ovarian tissue as possible in the hope that it may subsequently function normally

Kullander (1952) treated 67 women with secondary amenorrhoea with total doses of from 5 000 to 10 000 i u of pregnant mare serum and from 2 000 to 5 000 i u of chorionic gonadotrophin with the result that 30 subsequently had ovulatory cycles and 12 became pregnant It should be noted however that of 5 untreated patients 2 subsequently ovulated and 1 became pregnant

It is important to estimate the urinary gonadotrophins before embarking upon gonadotrophin therapy for it should be applied only to cases in which they are low or absent

Cortisone therapy

Based on the observations of Wilkins *et al* (1952) that in congenital adrenal hyperplasia cortisone therapy feminizes the patients and induces normal ovulatory cycles the idea has been applied to the amenorrhoea and menstrual disorders in women with simple hirsutism Jones *et al* (1953) treated 8 patients with follicular phase defects abnormalities of the proliferative phase and of ovulation or both with 50 milligrams cortisone daily for a month and 25 milligrams daily thereafter These patients were suffering from amenorrhoea oligomenorrhoea or anovulatory sterility and were moderately obese some had acne Their 17 ketosteroid levels were at the upper limits of normal or slightly raised In every case ovulatory cycles resulted and the 17 ketosteroid level fell In 2 cases the remission was permanent and 3 of the 5 married women became pregnant

it difficult to get the correct timing with such a regime because the cycles are often shorter than expected He believes that a more effective diminution of menstrual loss can be obtained by giving androgen continuously for 2 months and that with this plan the loss remains tolerable for a number of subsequent cycles

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been the author s experience nor that the treatment if prolonged causes any permanent damage to the mechanism of the pituitary control of the ovary

Other methods have been suggested Filler (1951) advocated 30 milligrams methyltestosterone daily during the 6 days before ovulation and was successful in all of 34 cases Hughes (1954) has used androgen therapy during the ovulatory phase but prefers oestrogen suppression of ovulation Kupperman and Goodman (1953) reported on androgen therapy during the ovulatory period giving 30 milli grams methyltestosterone or 50 milligrams methylandrostenediol for 6 or 8 days starting on the eighth to tenth day of the cycle with excellent results in 43 of 49 patients Ovulation was not inhibited and the mechanism of the therapy was not clear

ENDOMETRIOSIS

Androgen therapy

The first reports of the endocrine treatment of endometriosis were confined to the use of androgens Hirst (1943) summarized the literature up to that time and advocated this type of treatment in advanced cases in which radical surgery was contra indicated In 1947 he recommended injections of from 150 to 225 milli grams of testosterone propionate over a period of 2–3 weeks followed by oral methyltestosterone in daily doses of 10 milligrams for up to 3 or 4 years Green blatt (1952) preferred pellet implantation in the form of three 75 milligram pellets of testosterone and recommended keeping the patient under the influence of androgens for up to 2½ years despite the appearance of hirsutism acne enlarge ment of the clitoris and deepening of the voice It is claimed that this treatment reduces the pain and tenderness and the actual size of large cystic ovarian endo metriomas It is known that androgen administration will eventually lead to atrophy of the normal endometrium and presumably that is how it brings about the diminution in size of the chocolate cysts It should be emphasized however that this atrophy is only temporary and that endometrial growth will recur when therapy is discontinued The virilization which accompanies prolonged androgen therapy is clearly a serious disadvantage

Oestrogen therapy

Recently oestrogen therapy has been a more popular alternative and with large doses of stilboestrol as advocated by Karnaky (1948) The rationale for such treatment is not clear though some workers claim that by its suppression of gonado trophin release from the pituitary ovarian stimulation ceases the ovary atrophies the endometrium is deprived of the influence of ovarian hormones and conse quently both normal and ectopic endometrial tissue shrinks away Some writers say that this effect is permanent and that oestrogen therapy can cure endometriosis If so large doses are unnecessary 2 milligrams stilboestrol daily in the first half of the cycle would suffice Others believe that the beneficial effects of oestrogens or for that matter androgens are merely due to inhibition of ovulation and prevention of dysmenorrhoea which is a classical though by no means constant feature of endometriosis It has also been pointed out that the dysmenorrhoea dyspareunia and pelvic pain which sometimes accompany endometriosis may be due to the increasing development of adhesions In this case it would be illogical

Greenblatt (1953) treated 27 hirsute patients with or without menstrual irregularity with 50 milligrams cortisone continuously for from 2 to 18 months Thirteen had phases of amenorrhoea and there was marked improvement in the frequency of ovulatory menstruation in 6 2 of whom became pregnant Six had irregular menstruation which eventually became more regular in 4

At present there are few reports of the treatment of simple hirsutism and anovulatory infertility with cortisone and it would be dangerous to draw too close an analogy between this picture and that of congenital adrenal hyperplasia In the latter there is evidence of gross disturbance in the biosynthesis of adrenocortical steroids with inadequate production of glucocorticoids so that cortisone therapy is rational In simple hirsutism little if any abnormality of steroid metabolism has so far been discovered and cortisone therapy must be regarded as empirical

Deep radiotherapy

Though it is not strictly within the terms of reference of this chapter a word must be said concerning low dosage radiotherapy to the pituitary and to the ovaries or to both to induce ovulation The general impression is that this method of approach is more promising than endocrine therapy Most authors however would agree that it should be employed only as a last resort because of the possibility of inducing permanent amenorrhoea mutation of genes or congenital abnormalities in succeeding generations of patients so treated So far there is no evidence of congenital abnormalities having resulted even though some of the early patients treated are now grandmothers

TREATMENT OF SPASMODIC DYSMENORRHOEA

It is important to distinguish between primary spasmodic dysmenorrhoea and the secondary or congestive type associated with pelvic lesions There has been some tendency to believe that spasmodic dysmenorrhoea is a psychosomatic disorder The studies of Schuck (1951) in the health records at New York University of 300 students with dysmenorrhoea and 300 students who experienced no menstrual pain showed that there was no more evidence of psychoneurotic tendencies in the first than in the second group The actual cause of the pain however is still in doubt though there is some evidence to suggest that it is due to myometrial angiospasm

It seems to be established that spasmodic dysmenorrhoea does not occur in ovulatory cycles that is in the presence of progesterone and that suppression of ovulation leads to painless menstruation in that cycle Therapeutically moderate doses of oestrogen during the pre ovulatory phase will accomplish this In the author s experience 2 milligrams stilboestrol daily or 5 milligrams of equine conjugated oestrogen as a substitute if stilboestrol produces nausea is an adequate dose Treatment should commence within the first 5 days of the cycle and be continued for 14 days At least a week s interval should intervene between successive courses and if menstruation does not occur within this week it should be awaited before the next course is commenced This treatment has been completely successful in 87 per cent of the cycles in which it has been employed (Bishop and Orti 1952) Criticism has been levelled at the method on the grounds that it is only effective for a few cycles after which ovulation breaks through This has not

well being which cannot be obtained from the oestrogen alone and that undesirable side-effects are avoided because those from oestrogen and androgen neutralize each other out Thus it is claimed that there is less tendency to uterine bleeding and that the virilizing side effects of androgen are offset Numerous reports on the efficacy of this hormone combination are available and three may be singled out describing the results of blind clinical trials In the first by Greenblatt *et al* (1950) four preparations were available for 102 menopausal women They were eventually revealed as 0 25 milligram stilboestrol 0 25 milligram stilboestrol with 5 milligrams methyltestosterone 5 milligrams methyltestosterone and a placebo The combination of oestrogen and androgen produced the best effects though uterine bleeding and signs of virilism occurred in some cases In the second trial by Glass and Shapiro (1951) the combination of 5 milligrams methyltestosterone and 0 25 milligram stilboestrol was preferred by 72 per cent of 92 menopausal patients to each of the hormones given separately In the third trial by de Watteville and Lunefeld (1953) the combination of 0 01 milligram ethinyloestradiol and 5 milligrams methyltestosterone was more effective than either hormone given separately or than 25 milligrams methylandrostenediol by itself The author s preference is for an oestrogen alone rather than for a combination of oestrogen and androgen Hirsutism and acne have been problems in some of the cases he has treated with the current preparations of combined hormones on the market

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to expect any alleviation from endocrine therapy Novak has pointed out on many occasions that no endocrine therapy can be expected to cure endometriosis and that in many cases where such cures have been claimed the diagnosis has not been substantiated by actual palpation of nodules of endometriomatous tissue but has been assumed on account of the association of pelvic discomfort dysmenorrhoea dyspareunia and some degree of adnexal fixation In his view dysmenorrhoea is a rather rare accompaniment of endometriosis

It will be seen therefore that there is considerable difference of opinion as to the value of endocrine therapy in endometriosis The results in a few reported series are as follows

Fallas (1951) found that 58 of 91 patients in his series complained of some type of pain other than menstrual and that just over a third suffered from severe dysmenorrhoea He believed that in the majority the discomfort was due to massive adhesions for which hormone therapy could do no more in its advanced stages than possibly arrest the process Wallace (1951) analysing 45 proved cases found that the symptoms could sometimes be controlled by the simple measures used in the treatment of dysmenorrhoea that androgen therapy is sometimes palliative but never curative and that stilboestrol given in one case for 6 months caused complete fibrosis of the endometrial implants Hurxthal and Smith (1952) believed that large doses of stilboestrol could by inhibition of the pituitary gonadotrophins cause ovarian atrophy and lead to shrinkage of uterine fibroids and endometriomas They treated cases of endometriosis with daily doses of from 5 to 50 milligrams of stilboestrol Although relief of symptoms was satisfactory in some cases the unpleasant side effects seriously detracted from this treatment Finally Haskins and Woolf (1955) impressed by the fact that endometriosis undergoes remission during pregnancy and that this remission may persist for a long time attempted to imitate the endocrine activities of pregnancy by giving stilboestrol in increasing doses until 100 milligrams daily was being administered This dose was maintained for 3 months and then decreased by about 6 milligrams daily until it was finally withdrawn The subjective and objective responses were similar to those of pregnancy The endometriotic lesions became softer and less tender but did not change in size the remission might last for 18 months or more

THE MENOPAUSAL SYNDROME

The trends during the last few years have been to treat fewer menopausal women with hormone preparations to use smaller doses of oestrogen and to employ combinations of oestrogen and androgen Buxton (1951) used daily doses of 0 1 milligram stilboestrol increasing up to 0 5 milligram if the hot flushes were not controlled The author has for many years not exceeded 1 milligram stilboestrol (or its equivalent) daily and has usually found that 0 1 milligram twice or three times daily is the optimal dose The use of these small doses is not only more effective but it usually avoids the unpleasant side effects It has been the author s rule not to administer oestrogen constantly for long periods but to discontinue treatment every 3 to 6 weeks for a week or 10 days If this is not done the endometrium tends to become hyperplastic and uterine haemorrhage to occur when it will be necessary to perform a diagnostic curettage to exclude carcinoma

The use of combinations of oestrogen and androgen instead of oestrogen alone has been the most marked change in menopausal treatment over the last 6 years It is considered that the anabolic properties of the androgen induce a feeling of

well being which cannot be obtained from the oestrogen alone and that undesirable side effects are avoided because those from oestrogen and androgen neutralize each other out Thus it is claimed that there is less tendency to uterine bleeding and that the virilizing side effects of androgen are offset Numerous reports on the efficacy of this hormone combination are available and three may be singled out describing the results of blind clinical trials In the first by Greenblatt *et al* (1950) four preparations were available for 102 menopausal women They were eventually revealed as 0 25 milligram stilboestrol 0 25 milligram stilboestrol with 5 milligrams methyltestosterone 5 milligrams methyltestosterone and a placebo The combination of oestrogen and androgen produced the best effects though uterine bleeding and signs of virilism occurred in some cases In the second trial by Glass and Shapiro (1951) the combination of 5 milligrams methyltestosterone and 0 25 milligram stilboestrol was preferred by 72 per cent of 92 menopausal patients to each of the hormones given separately In the third trial by de Watteville and Lunefeld (1953) the combination of 0 01 milligram ethinyloestradiol and 5 milligrams methyltestosterone was more effective than either hormone given separately or than 25 milligrams methylandrostenediol by itself The author s preference is for an oestrogen alone rather than for a combination of oestrogen and androgen Hirsutism and acne have been problems in some of the cases he has treated with the current preparations of combined hormones on the market

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CHAPTER 19

THE ROLE OF ENDOCRINE FACTORS IN INFERTILITY IN THE FEMALE

KENNETH BOWES

GENERAL CONSIDERATIONS

IN VIEW of advances in the knowledge of reproductive physiology and of the intimate connection between the genital and endocrine systems it may seem paradoxical that in the clinical approach to infertility there is such a small group of women in whom endocrine treatment is beneficial But the limited help gained from such treatment is merely an example of the inefficacy of treatment of in fertility in general at the present time

Several important papers by Sharman (1947 1952) Stallworthy (1948) Jeffcoate (1951 1954) Bender (1952) and Buxton and Southam (1955) dealing with general considerations should first be mentioned Bender for example in a follow up study of 700 cases of primary infertility considered that only about one half of the pregnancies which followed investigation could be attributed to treatment Buxton and Southam were able to follow up 1 213 couples out of a total of 1 607 Of those in whom no obvious cause could be found on physical examination 37 3 per cent became pregnant as compared with 35 9 per cent of those with some demonstrable physical abnormality In their series 244 women were deemed to have ovarian dysfunction Of 147 women receiving treatment 31 3 per cent became pregnant Of those untreated (97) 51 2 per cent became pregnant Sharman (1947) showed that of 113 women with a normal uterus 66 6 per cent conceived as compared with 70 5 per cent of 147 patients with a hypoplastic uterus

Various estimates have been made as to the proportion of female infertility which can be assigned to endocrine causes These demonstrate themselves either as some disturbance of menstrual function or anovulation or less commonly as a definite endocrine syndrome of pituitary ovarian or adrenal origin Mazer and Israel (1941) found 24 6 per cent of infertility associated with menstrual distur bances Guerrero (1946) found a hormonal basis in 23 per cent Tyler (1949) in 20–25 per cent Johnson and Marshall (1950) in 20 9 per cent Buxton and Southam (1955) *in 20 1 per cent and Simard (1954) in 13 1 per cent Turning to more* specific conditions Jeffcoate (1954) assessed persistent anovulation as being present in less than 4 per cent of cases and Uriegar and Guerrero (1947) in 5 7 per cent McGoogan (1954) surveyed several series from the literature and found bilateral polycystic ovarian disease in only 25 cases out of 1 776 childless women (1 4 per cent) In his own series of 1 032 patients he found endometriosis in 2 9 per cent and polycystic ovarian disease in 0 58 per cent Turner *et al* (1951) reported 23 cases of endometriosis in 500 cases Frank (1950) noted 1 5 per cent of endo metriosis and 1 5 per cent of polycystic ovarian disease in his series Thus in

clinical practice major endocrine causes of infertility in the female are found in a very small fraction of cases and the group is probably no bigger than that due to failure to consummate marriage through ignorance

Looking at it the other way round the percentage rate of infertility in certain individual conditions is very high Stein (1950) stated that 85 per cent of his patients with polycystic ovaries complained of sterility as their first complaint and the infertility rate in endometriosis has been assessed at between 20–66 per cent (McGoogan 1954) Rubin (1947) *considered that functional sterility* existed in 94–96 per cent of cases of secondary amenorrhoea Day and Smith (1950) at the Mayo Clinic examined the reproductive histories of patients with ovarian dys function and found that of 425 such women 55 8 per cent had been unable to con ceive during a 6 year period

ALTERATION OF GENITAL FUNCTION

The physiological unit of the genital system consists of the uterus the ovaries and the anterior part of the pituitary gland Disorders or expression of disorder of an endocrine nature may affect any of the three components and diagnostic investiga tions are required to estimate at which level the disorder is occurring In turn the endocrine adjustments of the anterior pituitary and the ovaries are related to the functioning of other endocrine glands notably the adrenal and the thyroid and also to the functioning of the hypothalamus and the general metabolism of the person The uterus and ovaries are also pelvic organs and may themselves be subject to pathological processes which affect their functioning

Observations on each of the three ways in which genital function may be altered will be relevant to the discussion of infertility

The essential functional unit

During the past fifty years the co ordinated changes in the menstrual cycle have been extensively studied and the general pattern of activity between pituitary ovaries and uterus defined These need not be discussed here except to mention *that the ovarian steroids produce important metabolic changes in the endometrium* besides the usually described cyclical cellular histological appearances The changes in carbohydrate metabolism are important as glycogen is necessary to provide early nutrition for the developing embryo Large amounts of glycogen are normally present in the postovulatory secretory phase the source is glucose synthesis to glycogen being accomplished from a hexose phosphate split by a phosphate splitting enzyme (alkaline phosphatase) Atkinson and Engle (1947) showed that the alkaline phosphatase activity is high in the oestrogen phase of the cycle is reduced in the postovulation phase and disappears premenstrually This enzyme is also concerned in the metabolism of nucleoprotein and protein synthesis during the period of growth stimulation of the glands The significance of these metabolic changes has been shown by Hughes (1945) who estimated the glycogen and enzyme content in relation to the reproductive histories of patients and cor related disturbances of metabolism with miscarriage

In general disturbances in the function of the essential functioning unit can be considered as either follicular or luteal phase defects *These result in menstrual* symptoms and disorders or failure of ovulation which as Mazer and Israel (1941)

remarked merge imperceptibly into sterility Besides these major variations others occur pointing to differences in the quality of differing ovulatory and menstrual cycles Basal temperature records show that successive cycles vary in the type and degree of temperature shift and Tompkins (1945) has noted that conception is more likely to take place if there is an abrupt rise Cooperman (1949) has shown that during the first months of the menarche in girls temperature curves differ from those in the normal adult and in non ovulatory cycles Zuckerman (1941) pointed out that in the monkey the effect of an injection of oestrogen was not confined to the next period only but affected the endometrial state for the following period too The implications of this in the human were pointed out by Kaiser (1948) Brewer and Jones (1947) in their studies on the histology of the human corpus luteum and concomitant endometrial changes remarked on variations both in the type of corpus luteum and endometrium

Follicular phase defects

Follicular phase defects may affect the rhythmic alteration in the secretions of the cervix and so be a contributory cause of infertility Originally described by Seguy and Vimeux (1933) and Seguy and Simmonet (1933) these changes have received much attention Though it is generally agreed (Wheeler and Danziger 1955) that cytological variations in the cervical mucosa are insignificant the secreted mucus alters considerably in amount viscosity and reaction The secretion is strongly metachromatic and contains substances utilizable by sperma tozoa Without these substances the mobility of the spermatozoa is lost in a short time Postmenstrually and premenstrually the mucus is tough and cellular but at the mid cycle it is freer less cellular translucent and of lower viscosity Rydberg (1948) showed that during the pre ovular phase mucus taken from the cervix and spread on a slide will crystallize giving a fern like pattern This reaction increases in intensity and reaches its height at ovulation The crystallization results from sodium chloride in the mucus Bergmann (1949) showed that at mid cycle the mucus changes from gel to sol form the latter easily penetrated by spermatozoa In the postovulation phase the crystalline pattern disappears owing to the influence of progesterone

Inter relationship between the hypothalamus endocrine and genital systems

The hypothalamus and the cerebral cortex

The inter reactions between the various endocrine glands and their control have become increasingly important and the correlation between the humoral endocrine and autonomic nervous systems extensively studied as has the role of the hypo thalamus in the integration of these systems Green and Harris (1947) and Harris (1949) have demonstrated hypophyseal portal vessels along the pituitary stalk between hypothalamus and hypophysis and shown that the blood flow is from the former to the latter They suggested that the anterior pituitary might be influenced by the thalamus via these vascular channels Harris (1950) reviewing experiments in regard to this link described how electrical stimulation of the tuber cinereum in rabbits leads to ovulation though this cannot be produced by stimulation of the anterior pituitary If the stalk is severed the oestrous cycle in rats disappears Harris *et al* have also shown that in rabbits corticotrophin is secreted by stimulation of the tuber cinereum

Le Gros Clark and Meyer (1950) reviewed the anatomical connexions between cerebral cortex and hypothalamus and pointed out that between the cortical and autonomic levels of the nervous system they are much more definite than had been formerly realized They are both afferent and efferent in type the afferent areas are in the thalamic nuclei and the efferent fibres from the frontal region of the cortex They provide the neural relationship between the primitive emotional level of thalamic sensation and the higher cortical functions

Psychological factors and the genital system

Anatomical and physiological support is thus being found for the often observed psychological manifestations in clinical gynaecology and it emphasizes the impor tance of considering the individual as a whole The rhythm of the menstrual cycle is often affected by psychological factors and such disturbances of function may contribute to or cause infertility O Neil (1950) gave a general summary of the psychological manifestations in gynaecology and stressed their frequency also that there may be either primary or secondary factors and that often the psychological treatment required is of an essentially simple nature depending mainly on sympathy and guidance Blaikley (1949) and Wrigley (1949) have both been impressed with the psychological aspects of abnormal menstrual function Loeser (1943) studied cases of the sudden onset of amenorrhoea in women who had experienced bombing in London He found that the endometrium was of an arrested pre ovular type similar to a 16–18 day phase although the interval had been 38 days Whitacre and Barrera (1944) pointed out that the amenorrhoea experienced by 125 women out of 1 042 adults of menstrual age who were interned in Manila started abruptly after the first bombing there and before nutritional factors could have had any effect Fried *et al* (1951) gave further evidence of psychological factors affecting the genital system in an analysis of patients with pseudocyesis and in whom luteal activity of the ovaries was prolonged They considered that this finding was due to psychological factors acting through the hypothalamus and affecting the secretion of luteotrophin

Kluver and Bartelmez (1951) have described a Rhesus monkey which had been the subject of various neurosurgical procedures including temporal lobectomy Some 4 years after surgery it died during menstruation and in necropsy pronounced changes of abdominal and pelvic endometriosis were found It had previously shown other sex changes which were considered to have been secondary to hypo thalamic damage from operation Previously disturbances in pregnancy and labour had been noted in animals following experimental thalamic lesions (Dey *et al* 1941) and Theobald (1936) wrote of menstrual function in relation to the hypothalamus Reifenstein (1946) referred to hypothalamic disturbance as a common cause of amenorrhoea and Klinefelter Albright and Griswold coined the term hypothalamic amenorrhoea for a syndrome in which latent or manifest psychological trauma was thought to prevent the release of luteinizing hormone from the pituitary

Psychological origin of infertility

The question arises as to the possibility of a psychological origin of infertility and as a direct as opposed to a secondary phenomenon from alteration of the menstrual cycle Dunbar (1946) suggested that over anxiety to conceive might

result in a premature maturation of follicles and the discharge of ova not ready for fertilization she referred to this in connexion with the well recognized phenomenon of conception occurring 15-20 years after marriage She attributed such a result to change in emotional attitude and dispersal of emotional tension Kamman (1946) has referred too to the efficacy of adoption as a method of induced con ception in such cases of infertility On the other hand Hanson and Rock (1950) fully reviewed the literature on this fascinating angle of the subject and concluded that any beneficial aspects of adoption in relation to subsequent conception were speculative and without proof They followed up 202 couples who had adopted children In 15 (8 per cent) a subsequent pregnancy followed a figure within the possibilities of a spontaneous event and without adoption In only 4 of the 15 cases did the authors consider that pregnancy could undoubtedly be attributed to relief from emotional tone

Other endocrine glands

The normal functioning of the ovary and the pituitary may be considerably affected by other endocrine glands particularly the thyroid adrenals and the pancreas The mechanism of damping down or suppression of ovarian activity is not yet fully understood Clinically menstrual abnormalities are well recognized in cases of thyroid disease as is also the occurrence of infertility in both hyper function and hypofunction of this gland The relationship between thyroid and ovary can theoretically be affected by an alteration in the level of thyrotrophic activity of the pituitary by the direct metabolic action of the thyroid hormone at cellular level or by combined action with the adrenal Chu (1944) showed that ovulation did not occur in the rabbit following coitus if the thyroid had been removed but did so if chorionic or pituitary gonadotrophins were then given In these thyroidectomized animals follicle stimulating hormone was produced in excess and the ovaries became studded with multiple follicles which disappeared on their being given thyroid Engle (1944) noted in monkeys that thyroidectomy raised the oestrogen threshold level for bleeding and that thyroid restored it to normal Beirwalter and Bishop (1954) found that ketosteroid excretion was decreased in myxoedema but could be restored to normal with thyroid so that alteration in this adrenal function had occurred The inter relation between thyroid and adrenal in their effects on genital function had also been demonstrated by Gilbert and Gillman (1954) They found that oestrogen withdrawal bleeding did not occur in the hypophysectomized baboon although the uterus and sex skin were sensitive to oestrogens They believed that active thyroid function as well as adrenal function were both necessary for the promotion of endometrial bleeding

Intense research on the adrenogenital syndrome has drawn attention to the suppression of ovarian activity which results from the adrenal androgenic hormones In this syndrome the reticular zone tissue of the adrenal is hyperactive whereas the fasciculate zone is defective Several theories have been advanced to explain the effects on the ovary and the endometrium Wilkins *et al* (1952) believed that the excess androgens act via the anterior pituitary to depress gonadotrophin production with resulting ovarian failure This theory has been the basis of corti sone administration to patients with amenorrhoea hirsutism and infertility deemed of adrenal origin The cortisone is considered to act as a chemical

brake (*Wilkins*) excess androgen secretion is damped down pituitary gonado trophins are released and ovulation returns Others however consider that cortisone acts at end organ level by interfering directly with the circulating androgens

PATHOLOGICAL CHANGES IN THE OVARIES

Ovarian function may be affected by disease processes in the pelvis Inflammatory lesions may involve the ovaries and the function of ovulation be interfered with by the resulting adhesions Hyperplasia of the endometrium may then develop secondarily

Endometriosis involving the ovaries is frequently accompanied by impaired fertility or sterility This condition is dependent for its continuance at least and probably for its initiation on the continued action of the ovarian hormones The accompanying infertility rate is about 50 per cent

Effect on fertility of unilateral oophorectomy

The effect on fertility of the removal of one ovary is a subject on which little literature is available The general view is that unilateral removal is followed by stimulation of the remaining ovary to increased function Roman (1947) showed that this does occur in animals and Jeffcoate (1954) suggested that if one fallopian tube has to be removed for some reason the corresponding ovary should also be removed to ensure ovulation only from the remaining ovary whi h is near its own tube That the remaining ovary functions satisfactorily in the majority of cases is supported by the clinical investigation of Whitelaw (1951) who studied the occurrence of ovulation as judged by basal temperature records and endo metrial biopsies in 42 patients under the age of 30 years who had been subjected to unilateral oophorectomy He made his study the more valuable by using a control series of women with intact ovaries From the women operated on 164 out of 168 endometrial biopsies showed normal secretory endometrium Three women had minimal ovulatory changes and only one failed to show ovulation she developed menopausal symptoms within one year Of the controls 172 showed a normal secretory endometrium 2 showed no secretion and 8 an endo metrium of mixed pattern Compared with the normal figure of 5 per cent for non ovulation Whitelaw s figures were only 0 96 per cent in his unilateral oophorectomy series

Such satisfactory results however do not justify the free sacrifice of ovaries as one never knows whether a second operation may have to be performed for removal of the remaining ovary It is also difficult to forecast the likelihood of the menopause supervening Caffier (1937) for example followed up 174 women who had had one ovary removed The periods were altered in about two thirds of the cases and only 35 2 per cent subsequently became pregnant though 88 5 per cent had been pregnant prior to removal of the ovary Runge (1935) and Schmid Burgk (1938) have also studied ovarian function after operation

The Stein Leventhal syndrome

This condition of polycystic ovarian enlargement associated with amenorrhoea and infertility deserves separate mention As yet the aetiology has not been

determined Stein (1929) reported on the wedge resection of ovaries enlarged by multiple small cysts and recorded that the result was a resumption of menstruation Since Stein and Leventhal (1935) described the polycystic ovarian syndrome many references have been made to the condition Bailey (1937) reported several cases in the United Kingdom but most interest seems to have been taken in the United States of America Stein *et al* (1949) were able to report on 75 cases Meaker (1950) on 65 Auer (1951) on 10 Leventhal and Cohen (1951) on 10 Siegler (1952) on 10 and Haas and Riley (1955) on 12 cases There have been other papers by Leventhal (1941) Geist and Gaines (1942) Stein (1945) Reycraft (1949) Ingersoll and McDermott (1950) and Shippel (1955)

Diagnosis

The condition is usually diagnosed by a combination of symptoms and signs not all of which may be present in any individual case Classically there is secondary amenorrhoea with infertility if the woman is married frequently slight hirsutism and on vaginal examination one or both ovaries may be enlarged though not grossly and they feel of cystic or rubbery consistency Laparotomy confirms the enlargement of the ovaries to about two and a half times their normal size the capsule is thickened with mottling from the underlying small cysts (oyster ovary) Because of these appearances and the thecal hyperplasia the term hyperthecosis has been coined Microscopically follicles are found in all stages of development and are surrounded by thecal hyperplasia the thecal cells being luteinized Luteinized thecal cells may also be found in the ovarian stroma The urinary 17 ketosteroids are either at a normal level or slightly raised Pituitary gonadotrophin and oestrogen excretion levels are normal

When a definite diagnosis can be made the decision for laparotomy is easily reached but there may be difficulties owing to the symptoms and signs not all being present Primary amenorrhoea or amenorrhoea followed by menorrhagia or menorrhagia may be the accompanying menstrual disorders rather than the typical secondary amenorrhoea Sometimes hirsutism may not be present and bilateral ovarian enlargement not detectable Considerable care has to be taken in arriving at a diagnosis and some authorities employ accessory means of investiga tion such as gynaecography or coelioscopy in doubtful cases

Pituitary origin of condition

The Stein Leventhal syndrome still lacks a satisfactory explanation of its aetiology and the eventual solution should throw much light on ovarian function and endocrine relationships At the moment the view that the ovarian changes are secondary to disordered action of the anterior pituitary holds the field The anterior pituitary is considered to stimulate the ovarian follicles and the thickened capsule prevents their further development (the mechanical blockade of Leventhal (1941)) The hirsutism and menstrual changes are variously ascribed to oestrogen androgen imbalance to excessive production of progesterone or to excessive production of pituitary luteinizing hormone Shippel (1955) found difficulty in accepting the pituitary origin of the condition and attempted to show that the syndrome was an isolated part of a much bigger process He considered that the ovary was the source of the condition and that over growth or persistence of the theca cells occurred either from factors inherent in the ovary or locally

irritative to it He views the syndrome as one in which the woman may have varying menstrual symptoms over a course of years and gradually become de feminized The features which he regards as important are persistent anovulation from the time of menarche or pregnancy conditions which stimulate the reticulo endothelial system and local pelvic irritation

METHODS OF INVESTIGATION AND ASSESSMENT OF GENITAL FUNCTION

The number of investigations which theoretically can be employed in infertility far exceeds their value in practice In simple routine diagnosis it is necessary to combine the search for important common conditions with such tests as will show whether or not ovulation has occurred the normality of the endometrial response to ovarian hormones and exclude any obvious endocrine disease Thus a full history must be taken and physical examination made tubular insufflation per formed and an endometrial biopsy taken If necessary thyroid function should be determined and in some cases additional help can be obtained from examination of ovarian function by vaginal smears from radiological salpingography and from postcoital examination of the cervical mucus

Unless there is need for a more detailed endocrine investigation there is much to be said for not adding a multiplicity of tests as the constant repetition may increase any psychological tension present

Of the routine tests those directed to the occurrence of ovulation are important The only absolute proof of this is the finding of an ovum or its fertilization As yet we have no method of proving that true ovulation as distinct from follicular ripening and rupture has occurred and all methods of timing ovulation are presumptive Evidence can be obtained from direct observation of the ovary at laparotomy or coelioscopy indirectly by the study of the secondary effects presumptive of ovulation (biopsy of the endometrium vaginal cellular smears basal temperature records cervical mucus tests biochemical assays electric potential changes and biological tests of the Farris type on ovarian hyperaemia in the rat) Of these endometrial biopsy and basal temperature records are regarded as the most useful

Endometrial biopsy

An endometrial biopsy is usually taken during the premenstrual week though some prefer the first day of the period in spite of the accompanying autolytic changes Rock (1941) studied 400 cases and placed ovulation as occurring between 16–12 days before the onset of the next period Sporadic anovulatory cycles are not uncommon so that the repetition of a biopsy is necessary in several cycles to prove persistent anovulation warranting treatment The advantages of biopsy are that good presumptive evidence of ovulation is obtained and the inter reactions of the ovary and the endometrium can be assessed for as Hertig remarks post ovulatory effects are regular and progressive Disease such as endometrial tuber culosis can be excluded and the method may be used to evaluate the results or endocrine or antituberculous treatment Malkani and Chander (1954) summarized the value of this method as the one giving more information than any other single test and they divided endometrial responses into four categories hypo oestrogenic normal oestrogenic hyperoestrogenic and mature progestational changes

Basal temperature records

Endometrial biopsy gives better functional evidence than basal temperature records though the latter are now accepted as a simple method of demonstrating ovulation provided they are carefully taken and show the characteristic temperature shift from the lower level in the oestrogenic part of the cycle to the higher level in the latter half This shift is agreed to be due to progesterone influence on metabolism (Davis and Fugo 1948 Landau *et al* 1955) The interpretation of other than typical curves can vary widely and minor abnormalities should not be used as an excuse for ill judged therapy

The general trend today is to use methods of testing for ovulation as evidence of its occurrence rather than to try and estimate its timing accurately or to forecast its happening Many workers have shown how difficult it is to relate accurately the secondary phenomena of the menstrual cycle either to themselves or to ovulation Sturgis (1952) considered that there was no way to judge the time of ovulation within 24 hours There is therefore a limit to the use of temperature records as an aid to conception Hope deferred maketh the heart sick and there is no point in reducing hope further in the infertile by constantly drawing attention to function

The vaginal smear

The vaginal smear provides a simple test of oestrogenic function but the fact that many samples of cells have to be examined over a course of weeks restricts its use

Differential diagnosis of amenorrhoea

In cases in which amenorrhoea is the prominent symptom the level at which fault can be found is important in establishing a diagnosis Tests are employed to determine whether it is uterine ovarian or pituitary If the uterus is at fault bleeding will not occur when oestrogens are given but if withdrawal bleeding does result the defect is at a higher level Provided the ovaries are producing oestrogen bleeding will result when progesterone is given if it does not then either the ovaries are seriously affected or they are not reacting to stimulation by the anterior pituitary This can be tested by estimation of the follicle stimulating hormone level If this is less than normal the defect arises from changes in the pituitary if it is normal the amenorrhoea is probably arising from some general condition if the follicle stimulating hormone level is raised it is evidence that the ovary is at fault and the pituitary is trying ineffectually to stimulate it

Endocrine assays

These are needed in the investigation of some cases of amenorrhoea and in the diagnosis of hypogonadic function and of menstrual irregularities associated with signs of virilism or defeminization

Gonadotrophins

The value of urinary follicle stimulating hormone determinations in differentiating between amenorrhoea of ovarian and pituitary origin has been mentioned

Ovarian hormones

In infertility there has not been found much scope to date for the investigation

of the levels of these substances in body fluids Pregnanediol excretion has been used as an index of corpus luteum function but as it represents only a fraction of progesterone metabolism it has not proved of much practical value Newer methods of blood oestrogen determination are being tried but as a review in the British Medical Journal (1954) stated the time has not arrived for their clinical use

Urinary corticosteroids

The excretion of these substances has proved of considerable value in the diagnosis of adrenal conditions and clinically the test has been applied particularly to patients suffering from amenorrhoea and infertility with associated signs of viriliza tion In general some degree of virilization with normal or slightly elevated excretion of 17 ketosteroids (10–20 milligrams every 24 hours) is most likely to be due to ovarian disorder if the ketosteroid level is moderately increased (20–50 milligrams) adrenal hyperplasia is probable or a rare ovarian tumour of the Leydig like cells in the hilum or an arrhenoblastoma a high 17 ketosteroid level (50 milligrams or above) is likely to indicate an adrenal tumour

Other diagnostic methods

The difficulties sometimes experienced in the diagnosis of small ovarian swellings and of determining whether in fact the ovary is enlarged or not or whether it has an abnormal surface have led to the reintroduction of coelioscopy or as an alter native culdoscopy and laparotomy The former method has the advantage that no operation is performed though it should not be lightly undertaken as it can have complications Moreover it is not always possible to visualize the pelvic contents satisfactorily without making endoscopy an inefficient laparotomy Culdoscopy has been developed by Decker and Sherry (1944) Decker (1952) and his followers Palmer (1954) in a paper read to the Society for the Study of Fertility demonstrated the logical limits of these methods which he has used in over 2 000 cases MacFadyen *et al* (1952) reviewed the various methods of endoscopy used in 161 cases they prefer culdoscopy Of their patients 6 8 per cent had complications and in 13 per cent they failed to enter the peritoneal cavity In a suitable case laparotomy has the great advantage of permitting a full and considered exploration

Radiography should be mentioned as another method of investigation which can help in the diagnosis of ovarian pathology Either a pelvic pneumoperitoneum can be produced or it can be combined with hysterography to produce contrast By this method a very satisfactory visualization of the pelvic organs can be obtained

TREATMENT

The treatment of infertility due to endocrine factors has up to the present proved very disappointing Admittedly we expect to gain help in the worst cases for minor alterations may be temporary and cure themselves The passage of time an attitude of reasonable optimism provided no serious lesion is found and care as to the general health will probably be as effective and less unsettling than complicated schedules unless definite endocrine disease is present The criticism of the difficulty in assessing the effects of treatment must be emphasized here Sharman (1947) observed that there was no difference in a treated and untreated

series of patients with uterine hypoplasia Rabau (1945) similarly noted that the effects attributed to thyroid treatment were not significantly better than spontaneous cure but these were presumably not true cases of thyroid deficiency

At the present time observations on therapy can only be fragmentary as they are not capable of unification into a logical plan It is probably better to discuss the use of available methods rather than the treatment of a condition which may be due to so many different causes

Endocrine therapy

The gonadotrophins

These are of two varieties those derived from animal pituitary preparations and those of a chorionic type from human pregnancy urine or serum Their use would logically be for the induction of ovulation in patients who have been proved to have persistent anovulation and in whom ovarian failure as a cause has been excluded

Probably no single endocrine factor in sterility has been the target of so much well meant but empirical therapy as has anovulation If this condition is diagnosed as a persistent state all methods of treatment are unsatisfactory Spontaneous ovulation may occur apart from treatment and conception may result as is exemplified by the following case history

> A patient, aged 37 years, who had been married for 11 years had had no periods since her marriage and was anxious for a child Following rhythmic administration of oestrogens and ethisterone she had three or four uterine losses and then tired of her pills. The uterine losses ceased Four years later she was seen again this time pregnant having conceived during her continued amenorrhoea.

Animal pituitary preparations have several disadvantages They quickly give rise to antihormones they are difficult to purify and they have species differences It is now agreed (Sturgis 1955) that they seem to be of no use clinically Chorionic preparations are chiefly luteotrophic

The early hopes engendered by Davis and Koff (1938) who used pregnant mare s serum to induce ovulation have not been fulfilled and Brewer *et al* (1942) who used controlled groups of patients and confirmed by laparotomy failed to induce ovulation Many other workers have tried both types of preparation either separately sequentially or in combined form Hall (1947) Hamblen *et al* (1941) Rydberg and Pedersen Bjergaard (1943) Siegler and Fein (1939) and more recently Zondek have all published encouraging results from the use of either human pregnancy serum preparations or from the sequential administration of both follicle-stimulating and luteinizing hormones Other authors such as Geist *et al* (1941) and Erving *et al* (1940) have not been able to confirm ovulation after such treatment Siegler (1952) treated 31 patients 4 had ovulatory cycles after treatment ceased and 1 pregnancy resulted Finkler (1949) published the results of 76 patients suffering from secondary amenorrhoea treated by endocrine therapy gonadotrophins were given by the Rydberg method and if indicated ovarian steroids were also given Pregnancy occurred in 34 2 per cent of the cases and the author adopted a middle view of the value of this method The use of these substances is not without complications as allergic reactions can occur and the ovaries may become enlarged from the formation of lutein tumours (Movin 1949)

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which appeared to have improved as judged by this test It was interpreted as improved corpus luteum function However as other methods of treatment were being employed at the same time it is difficult to draw any sound conclusions Criticism of empirical thyroid therapy has been expressed by Rabau (1945) Tyler (1949) and Swyer (1955) and a paper pertinent to this issue by Buxton and Herrman (1954) showed by a careful study of a control series that thyroid was only of questionable value in these cases

The use of cortisone

Following the work of Wilkins *et al* (1952) who demonstrated the action of cortisone in congenital adrenal hyperplasia Jones *et al* (1953) and Jones and Jones (1954) have shown that cortisone can be used to correct the effects of adrenal hyperplasia in older patients and to restore menstrual function ovulation and as an aid to conception Suitable cases are those of postpuberty hirsutism infertility and amenorrhoea or oligomenorrhoea both with and without elevation of 17 ketosteroid excretion level Oral cortisone was used in a dosage of 50 milligrams daily for a month and then 25 milligrams daily subsequently Of the 16 patients who had a raised ketosteroid level all started to ovulate after treatment and 5 of the 10 married patients conceived Of the 15 patients with normal ketosteroid excretion all ovulated and 7 of the 11 married patients conceived

It appears therefore that in selected patients this form of treatment is valuable Another possible use of cortisone may be in the treatment of the Stein Leventhal syndrome as suggested by Benson Kolb and Traut (1955)

Irradiation of the pituitary and ovaries

The indifferent and uncertain results of the treatment of protracted amenorrhoea and infertility by hormones have led some workers to irradiate the pituitary the ovaries or of both glands as an alternative method The mode of action is not clear Some consider that stimulation of function takes place by either altering biochemical factors or by hyperaemia though the latter has not been observed at laparotomy Others attribute results to destruction of some of the follicles with the result that some of the remainder receive the gonadotrophic influence and mature A low kilovoltage is used and care is necessary as to dosage details and shielding

The employment of this method has led to considerable controversy A fair summary has been given by Collins (1950) who circularized 410 practising gynaecologists 70 per cent stated that they would not use the method and 30 per cent replied that they did and had used it for a total of some 4 200 patients and with no ill effects In individual series there were instances of complications

Irradiation as a method of producing ovulation was introduced by Beclere (1926) and Rongy (1924) Rubin who since 1926 had had much experience of it stated in 1954 that he was able to report that no less than 80 per cent of patients resumed regular menstruation and 50 per cent conceived he estimated that only 6–10 per cent of untreated patients with similar difficulties would have become pregnant spontaneously Kaplan (1954) reported 660 patients treated 270 became pregnant and had 347 normal children A considerable literature has now grown up on this subject but not all is in favour of its use Besides the authors mentioned above Drips (1948) Edeiken (1933) Finkler and Friedman (1938) Friedman and

A possible use of luteinizing hormone has been suggested by Brown and Bradbury (1947) They aimed at prolonging the action and life of the corpus luteum by administering this hormone in the postovulatory phase

To sum up gonadotrophins appear to have little clinical scope at the present time

The ovarian hormones

These substances may be used to influence either anterior pituitary action or the functioning of the target organ or the uterus in its endometrial myometrial or cervical components

Since Hamblen *et al* (1941a) suggested that the cyclical secretion of the gonado trophins was influenced by ovarian hormones an alternative method of attempting to induce ovulation has been tried Bishop (1950) suggested a smaller dosage of oestrogen than Hamblen with the idea of minimizing the risk of inhibition of ovulation Stilboestrol 0 5 milligram was given daily for 21 days and ethisterone 30 milligrams daily, for the last 7 days of a three weeks course Siegler (1952) using a variant of this method treated 36 patients ovulatory cycles started in 12 and 2 pregnancies resulted

In view of Sharman s comparative findings of pregnancies in patients with normal or infantile uteri there seems little point in treating hypoplasia of the uterus unless examination shows that there is coincident evidence of impaired ovarian function If the uterus is small and there is evidence of normal ovulation and ovarian function it will fail to respond to hormone treatment as there must be target end organ failure

Ovarian steroids can be used if endometrial function is deficient as revealed by biopsies Such a diagnosis should not be made on an abnormality of the basal temperature record alone Hughes *et al* (1950) showed the beneficial effects of oestrogens in small dosage (0 1 milligram stilboestrol for 12 days following the end of the menstrual period) in cases in which the glucose metabolism of the uterus was deficient

Ovarian hormone can also be used to influence the type of cervical secretion The free watery flow of mucus at the time of ovulation may not occur and post coital tests may show spermatozoa impeded in their passage through viscid pre ovulation secretion As this condition is probably more contributory than causal in infertility oestrogens are best given for a few days only prior to ovulation rather than throughout the pre ovular phase

The use of thyroid

Since its first exhibition for myxoedema and cretinism thyroid extract has had a favoured therapeutic history for it is a potent and simple substance to use For many years it has been employed empirically in certain cases of infertility and menstrual disorders and will probably continue to be so used in spite of the lack of real evidence for its efficacy in such conditions If there is proof of hypothyroid function then its use is essential but minor deviations of the basal metabolic rate or an over weight patient are not sufficient reasons for its exhibition Johnson and Bradbury (1953) employed ½ grain thyroid daily for protracted periods in selected patients and achieved 10 pregnancies in 22 married women out of 38 effectively followed up Of these 6 had pregnanediol assays made to assess ovarian function

more optimistic and considered that possibly 68 per cent of patients may conceive after operation The benefit of operation may derive from several effects Dyspareunia is relieved operation in earlier stages of the disease is more likely to be beneficial and it is possible that the removal of the diseased tissue leads to more normal functioning of the ovary

Wedge resection of the ovaries

There are now many reports of the use of a simple wedge resection of the ovaries in the Stein Leventhal syndrome The mode of action of this simple operation is unknown Some consider that by the removal of the tissue the few follicles remaining can be stimulated by follicle stimulating hormones (Leventhal 1941) others consider that incision of the ovarian capsule leads to an easier escape of the ova Shippel (1955) is of the opinion that the removal of thecal tissue is the rationale of benefit and that the ovaries can then react again to gonadotrophins Although wedge resection is usually performed an alternative method has been tried by Bailey (1937) who bisected the ovary and then extroverted it This would seem logical but it leads to adhesions

Whatever the explanation of the mechanism of wedge resection in this syndrome may be there seems no doubt that in properly selected cases the results are favourable both to the resumption of menstruation and possibilities of conception The accompanying hirsutism is little relieved Since the early papers by Stein and Leventhal (1935) and Bailey (1937) there have been other reports of the results of such treatment Stein *et al* (1949) reported on 75 cases so treated of whom 40 complained of sterility Of the latter 26 (65 per cent) conceived after operation and 89 per cent had regular periods Weinstein (1949) noted 11 pregnancies in 17 patients and Leventhal and Cohen (1951) 5 pregnancies in 6 patients Auer (1951) reported 7 out of 10 patients conceiving Siegler (1952) 6 out of 10 Meaker (1950) 66 per cent of 53 infertile patients conceiving and Haas and Riley (1955) that 4 out of 5 conceived within a few months The last authors summarize the criteria for selection of cases There must be acyclic ovarian function normal excretion levels of pituitary gonadotrophins the oestrogen excretion should be within normal limits and the ovaries should be questionably or definitely enlarged In favourable cases pregnancy follows soon after operation and patients should be encouraged to try to conceive as soon as possible because as Auer remarks the syndrome may recur

It is possible that in the future surgery will take a less emphatic place in the treatment of this syndrome Cortisone has already been tried As Shippel remarks notwithstanding the excellent results obtained with wedge resection in the treatment of the hyperthecosis syndrome it is felt that the future therapy lies in the direction of endocrinological depression of pituitary gonadotrophin production for a sufficient length of time to allow for involution of the theca to occur without at the same time jeopardizing the recovery of the ovaries

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Finkler (1942) Hanman (1947) Israel (1952) Mazer (1947) have published good results

Possibilities of genetic damage

There are several considerable disadvantages to the use of irradiation which restrict its use and make imperative very careful investigation before employing it The disadvantage which has received most attention is the possibility of genetic damage with the risk of danger to future generations Anxiety has been expressed about the effects of irradiation under modern living conditions (Medical Research Council 1956 National Academy of Science 1956) Gene mutation is increased by radiation This damage is irreparable and may take several generations to become manifest so that published series of a few generations are not a sufficient guide to the safety of the method Muller who since 1927 has been concerned with the genetic effects of induced mutations in the fruit fly considers the method unsafe (Muller 1954) as do other scientists such as Russell and Russell (1952) Crainz (1946) reviewed the literature of the subject from an experimental aspect Plants insects and small animals all showed the possibility of recessive gene mutations Rugh (1954) pointed out that the method has a doubtful basis for its action for 50 000 r can be given to the anterior pituitary without altering its function as judged by gonad stimulating effects It is difficult therefore to see why a thera peutic dose of only 80 r can have more than a psychological effect

Thus all theoretical evidence is against the use of this method of treatment although it is not possible to exclude a species difference in the human Ingalls *et al* (1955) summarize the matter succinctly when they remark that it may be employed if one can philosophically justify what may be a genetically perilous pursuit

If this treatment is to be considered certain contra indications should be borne in mind Irradiation should not be used in younger (under 20 years) or older (over 35 years) patients for fear of inducing permanent amenorrhoea and for the same reason a woman who has already had one ovary removed should not be so treated Asherman (1952) noted that 50 per cent of women over 35 years of age ceased to menstruate following irradiation Great care too must be taken that the patient is not pregnant for foetal abnormality has been shown to result

Surgical treatment

Exploration of the pelvis may be indicated in endometriosis and for wedge resection of the ovaries in the Stein Leventhal syndrome Occasionally the residua of a pelvic infection may have to be treated by separation of adhesions impeding ovarian function and to renew tubal patency Before laparotomy for any of these conditions careful diagnosis of the state and its extent is necessary and particularly if the Stein Leventhal syndrome is suspected In younger women endometriosis should be dealt with by conservative methods if possible Subsequent pregnancy though not following as often as is desired occurs more frequently than might be expected from the usual state of disorganization of the pelvic tissues Kelly and Schlademan (1949) reported 24 per cent of 38 patients with endometriosis who became pregnant after conservative operations Haas (1951) reported 40 per cent with a similar condition compared with 8 6 per cent in untreated cases He has given a useful review of the literature of this subject McGoogan (1954) was even

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CHAPTER 20

CARCINOID TUMOURS AND SEROTONIN

P J D Snow

INTRODUCTION

DURING THE last few years a new and curious syndrome has been recognized due largely to the observations of a group of Swedish workers Their interest was first aroused in 1951 when a boy of 19 years was admitted to the General Hospital Malmö with pulmonary stenosis and a curious flushing of the skin Autopsy confirmed the pulmonary stenosis but it also unexpectedly revealed a metastasizing carcinoid tumour (Biörck *et al* 1952) A few months later Waldenström and Ljungberg (1955) saw another patient with carcinoid metastases and a similar type of flushing These patients were described by Waldenström at a meeting of the Swedish Society of Internal Medicine in 1952 and at the same time a further case was contributed by the pathologists Four more cases were subsequently recog *nized in Sweden by the peculiar flushing alone and Thorson et al (1954) published* a full account of all these cases together with others they had collected from the literature They considered that the association of flushing diarrhoea and pul monary stenosis with a metastasizing carcinoid tumour in so many cases was unlikely to be fortuitous and that these features constituted a hitherto unrecognized syndrome

Meanwhile Isler and Hedinger (1953) of Zurich had also published reports of 3 patients with malignant carcinoids and pulmonary stenosis and had suggested that these abnormalities might be related Several other case reports followed these pioneer descriptions and a review of the earlier literature brought several more cases to light the earliest being Sir Maurice Cassidy s (1931) A particularly complete description of the syndrome was given by Millman (1943) but his experience was limited to this one case and he regarded the association as fortuitous

However unlikely it may have seemed that such a curious assortment of features could have a common denominator especially with such an apparently inert tumour as a carcinoid these collective case reports established a relationship beyond doubt The discovery by Lembeck (1953) that carcinoids secreted serotonin provided a possible explanation for the symptoms and there can be little doubt that the description of the carcinoid syndrome at a time when the properties of serotonin were being intensively investigated considerably increased the interest in both subjects

SEROTONIN (5 HYDROXYTRYPTAMINE)

The existence of serotonin was first suggested by the early observations that defibrinated blood contained a substance which caused vasoconstriction and often

brought perfusion experiments to an untimely end This effect could be prevented by preliminary perfusion through the lungs (Herrick and Markowitz 1929) an observation of added significance at the present time The numerous attempts to isolate and identify this substance ended in failure until Rapport *et al* (1947 1948a b) isolated a crystalline compound which they called serotonin and which subsequent investigation showed to be 5 hydroxytryptamine (5 HT) (Rapport 1949) 5 HT was subsequently synthesized first by Hamlin and Fischer (1951) and later by Speeter *et al* (1951) Asero *et al* (1952) and Harley Mason and Jackson (1954)

Meanwhile Erspamer *et al* had been attempting to isolate and identify the factor responsible for the histochemical properties of the enterochromaffin cells of the alimentary tract Vialli and Erspamer (1933) described a series of colour reactions and pharmacological effects for this substance which they called enter amine and its indolic nature was first suggested by Erspamer as early as 1946 With the aid of paper chromatography Erspamer and Boretti (1951) were able to separate enteramine from contaminants and it was identified as 5 HT by Erspamer and Asero (1952)

Since carcinoids are derived from enterochromaffin cells it was logical to assume that they might also be rich in 5 HT Feyrter and Unna (1937) isolated an unidentified substance from a carcinoid tumour but it remained for Lembeck (1953) to confirm the presence of large quantities of 5 HT in a carcinoid thus vindicating Masson s (1928) prophetic insistence that carcinoids were endocrine tumours It is not surprising therefore that Thorson *et al* (1954) suggested that an excess of circulating 5 HT might be responsible for the syndrome they described and confirmation that 5 HT was indeed produced in excess in these patients was provided by Pernow and Waldenstrom (1954) Page *et al* (1955) and Snow *et al* (1955) To what extent this finding explains the symptoms will be discussed later

Formation and metabolism

It has been shown that 5 HT is derived from dietary tryptophane by preliminary hydroxylation to 5 hydroxytryptophane (Udenfriend *et al* 1953) which is then decarboxylated to 5 HT (Clark *et al* 1954) The enzyme responsible for catalysing the first stage has been found in the liver and the kidney while the decarboxylase is more widely distributed (Gaddum and Giarman 1956) More recently, Sjoerd sma *et al* (1956b) found 5 hydroxytryptophane decarboxylase but not tryptophane hydroxylase in extracts of carcinoid tumour tissue They pointed out that the latter enzyme is difficult to demonstrate in any isolated tissue and that failure to find it does not necessarily imply its absence Smith *et al* (1957) have meanwhile found 5 hydroxytryptophane as well as 5 HT in the urine of a patient who had a carcinoid metastasis in the kidney This had almost certainly been formed by the tumour cells and it therefore seems likely that the whole synthesis of 5 HT from tryptophane can be carried out in the tumour itself and presumably in nor mal enterochromaffin cells too

5 HT is rapidly oxidized by amine oxidase which is present in the lung (Bradley *et al* 1950) the liver and the kidney (Blaschko and Philpot 1953) to 5 hydroxy indole acetic acid (5 HIAA) (Sjoerdsma *et al* 1955) which is excreted in the urine Other 5 hydroxyindoles are probably formed as well since Sjoerdsma *et al* (1956b) were only able to recover 80 per cent of 5 HT given to normal subjects as urinary

5-HIAA as many as 14 other indoles have been found in the urine of patients with carcinoids (Macfarlane *et al* 1956) A small proportion of the urinary 5 HIAA may not be derived from 5 HT (Blaschko and Hope 1955) but the enormous amount found in patients with carcinoids is undoubtedly due to the excessive secretion of 5 HT and provides a convenient and reasonably accurate index of 5 HT production

Using this index Sjoerdsma *et al* (1956b) have shown that in normal individuals only about 1 per cent of the dietary tryptophane is converted to 5 HT whereas

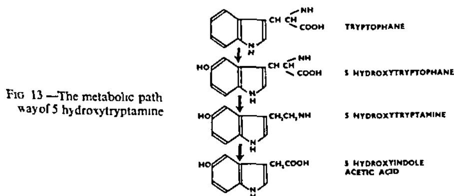


Fig 13 —The metabolic pathway of 5 hydroxytryptamine

this proportion may reach 60 per cent in patients with carcinoids Correspondingly less tryptophane is thus available for conversion to nicotinamide which may explain the occasional occurrence of pellagra in these patients (McNealy and Jones 1946 Currens *et al* 1945)

Pharmacological effects

The pharmacological effects of 5 HT have been intensively studied and full accounts have been given by Erspamer (1954) Page (1954) and Robson and Keele (1956) Unfortunately many of the effects vary with the preparation and animal used and results must be applied to man with caution Nevertheless some actions appear to be well established and help to explain the pathogenesis of certain features of the carcinoid syndrome

The vascular responses to 5 HT are especially variable under different conditions and in different species Acting directly on vessels it causes vasoconstriction but Page and McCubbin (1953) believed that inhibition of existing neurogenic vaso constriction is a more important effect and that depending on the degree of neuro genic control present the response may be pressor or depressor They suggested the term amphibaric to describe this variable response One of the most pro nounced effects of 5 HT is to increase alimentary peristalsis probably due to stimulation of postganglionic cholinergic fibres in the intestine (Gaddum and Hameed 1954) Injection of 5 HT intravenously or into the right auricle in cats caused both bronchoconstriction and pulmonary vasoconstriction with a conse quent rise in pulmonary artery pressure Injections into the pulmonary veins (Reid and Rand 1952) or left ventricle (Comroe *et al* 1953) on the other hand were without effect The renal effects of 5 HT have been studied by Erspamer

(1954) who claimed that it inhibited the diuresis of water loaded rats probably by afferent glomerular arteriolar constriction and believed that it was identical with the stable antidiuretic substance of Ginsburg and Heller (1951) Page (1954) and his colleagues however were only able to produce oliguria with hypotensive doses of 5 HT

There have been very few observations on the effects of 5 HT in man possibly due to the unpleasant sensations which may be produced On intravenous injection the symptoms range from nothing at all to tingling or pricking of the skin difficulty in breathing an urgent desire to empty bowels and bladder abdominal pains weakness nausea and a sensation of impending dissolution

The signs have included hyperpnoea and tachycardia The blood pressure response is variable but usually only slight in either direction (Page 1954) and blushing was observed by Page and McCubbin (1953) in several hypertensive patients who were given intravenous 5 HT

The distribution and functional significance

5 HT has been identified in the alimentary tract blood spleen and brain of vertebrates (Erspamer 1954 Amin *et al* 1954) the salivary glands of octopods the skin of amphibia (Erspamer 1954) the venom of wasps (but not of bees) (Jaques and Schachter 1954) in the trichomes of the plant Mucuna pruriens (Bowden *et al* 1954) and in the sting of nettles (Collier and Chesher 1956)

Normally practically all the 5 HT in the blood is present in the platelets (Humphrey and Jaques 1954) and Udenfriend and Weissbach (1954) suggested that the megakaryocytes may be the source of 5 HT However there is no evidence to support this suggestion and as Humphrey and Toh (1954) have shown that platelets avidly absorb 5 HT they suggested that the latter is formed in the entero chromaffin cells or elsewhere and subsequently taken up by the platelets Toh (1954) has produced evidence in support of this view The source of 5 HT in the central nervous system is at present unknown

The wide distribution of 5 HT coupled with its intense pharmacological activity suggests a role of considerable biological importance and Erspamer (1954) considered it to be a true hormone At the present time however there is no definite evidence that 5 HT has a physiological function although several have been suggested Correll *et al* (1952) suggested that by virtue of its vasoconstrictive properties it may play a part in securing haemostasis but Erspamer (1954) has put forward reasons for doubting this Page and McCubbin (1953) considered that it plays a part in the control of vasomotor tone but this suggestion has also been criticized by Erspamer (1954) who believed that its most important effect was on the kidney but this view has in turn been refuted by Abrahams and Pickford (1956) A common objection to all these suggestions is the normal absence of 5 HT from the plasma

The presence in and probable formation of 5 HT by the enterochromaffin cells suggests a possible action on intestinal activity Similarly the presence of 5 HT in the brain and the induction of schizophrenic states by 5 HT antagonists led Woolley and Shaw (1954) to suggest that it played an important part in nerve metabolism On the other hand Feldberg and Sherwood (1954) have found experimental evidence of behavioural effects from an excess of 5 HT in the brain

but only one patient with the carcinoid syndrome has so far been reported to have had a psychosis and even this was probably incidental (Sjoerdsma *et al* 1956b)

PATHOLOGY

The pathology of carcinoid tumours is adequately dealt with in standard text books but there are a few points which seem to require special mention in view of recent developments

Derivation

Carcinoids are tumours derived from the yellow Kultschitzky or enterochromaffin cells of the alimentary tract These cells take up both silver and chrome salts (hence the alternative names argentaffinoma and chromaffinoma) and are widely distributed throughout the alimentary tract from stomach to rectum they may also be found in the bile ducts and pancreas Their origin has never been satisfactorily settled but Danisch (1923) suggested largely on the basis of staining reactions a developmental relationship with the sympathetic ganglia and adrenal medulla Staining reactions alone are of course no proof of such relationship but the recently established secretory function of the chromaffin cells of the alimentary tract does bring them more into line with those of the adrenal medulla and the clinical similarity between phaechromocytomas and carcinoids is inescapable

Only metastasizing argentaffinomas—usually with numerous hepatic secondary deposits—appear to be capable of causing the syndrome presumably because the 5 HT output of the primary tumour alone is insufficient to have any effect Even extensive metastases do not necessarily cause symptoms and it has accordingly been suggested (Waldenstrom and Ljungberg 1955) that carcinoids may be derived from more than one type of chromaffin cell with consequent differences in the secretory activity of the tumour It is known that macroscopically some carcinoids appear yellow and others white and Jacobson (1954) distinguishes between argentaffinic and argyrophilic cells on the basis of staining reactions but there is no conclusive evidence that there is any difference between these cells as regards the secretion of 5 HT Speculation on the possibility of two distinct types of precursor cell may in fact be based on a fallacy for although not all metastasizing carcinoids are associated with the syndrome an increased secretion of 5 HT has been demonstrated in all patients in whom this investigation has been carried out whether symptoms were present or not

Other explanations must account for the failure of quite large quantities of 5 HT to have any apparent effect in some patients but unless further investigations show that some carcinoids do not secrete 5 HT there appears to be no need to postulate different types of tumour The admittedly wide variation in the output of 5 HT even of similar sized growths may have a simple explanation for it is only to be expected that the secretory activity of different tumours will vary depending perhaps on their degree of differentiation

Although the argentaffin granules are thought to be due to the presence of 5 HT or a related material (Barter and Pearse 1953) no attempt seems to have been made to correlate the secretory activity of the tumour with the histological appearances and it may be significant that of two cases seen by the author these granules were

numerous in the tumour cells of one patient with very severe symptoms and a high output of 5 HT but practically absent in the other who had very mild symptoms and only a slight increase in the 5 HT output

Malignancy of carcinoid tumours

Carcinoid tumours vary widely in their degree of malignancy even in those with undoubted metastases While some patients run the rapidly downhill course of most types of cancer others may live for 10 or 20 years with little change and a striking absence of cachexia Mallory (1940) for example described a patient in

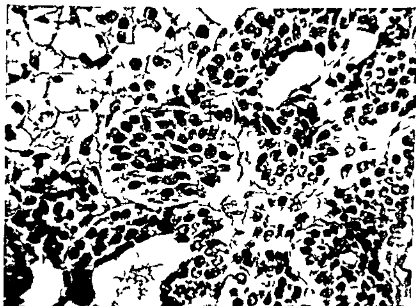


FIG 14 —Histological appearances of a carcinoid metastasis (Photo micrograph × 400)

whom carcinoid metastases found at laparotomy were unchanged at autopsy 20 years later and one of Waldenstrom s patients was still alive 10 years after metastases were first noted whilst another died 16 years after a laparotomy dis closing metastases To account for this unusually slow progression in some patients Waldenstrom and Ljungberg (1955) suggested that the tumour masses arise from multiple hyperplasia of the enterochromaffin cells rather than from true neoplasia with metastases a condition for which they propose the term carcinoidosis There is however no evidence to support this suggestion although it is known that carcinoids are often multiple (Foreman 1952) Karsner (1949) has stated that removal of the primary tumour may prevent progression of metastases but this belief may have arisen from the very slow progression of some of these tumours whether the primary growth remains or not Nevertheless it seems worth while to remove the latter whenever possible The most common site is the lower ileum and not as might be supposed in the appendix only about

4 per cent of the well known appendiceal carcinoids metastasize (King 1944) compared with 20 per cent of those from the ileum (Cooke 1931) Other primary sites include stomach jejenum Meckel s diverticulum colon rectum gall bladder pancreas ovarian teratoma and testis

One of the most puzzling features of the syndrome is the occurrence of pul monary and tricuspid valvular lesions Clinically the pulmonary valve appears to be most commonly affected but lesions of the tricuspid have been found to be equally common at autopsy Fibrous thickening of the valve cusps and shortening of the chordae tendineae are usually present but there is no indication of how these changes arise

CLINICAL FEATURES

The principal clinical features of the syndrome in the first reported cases included flushing diarrhoea valvular lesions of the right side of the heart enlargement of the liver and occasionally bronchospasm At the time of writing over 50 examples of the syndrome have been recorded enabling the relative frequency of these manifestations to be assessed Over 80 per cent of these patients had flushing 75 per cent had diarrhoea or colic 65 per cent had valvular lesions in the heart and 15 per cent had asthma The most constant feature of all however was hepatomegaly present in over 90 per cent of cases Since the symptoms apparently depend on the presence of a large bulk of secreting tumour tissue of which the liver is usually the main site this almost constant presence of hepatomegaly is understandable These clinical features will now be considered in detail

Cutaneous vascular phenomena

Although the term flushing has been used hitherto this is only one of several types of skin change some of which are permanent and others paroxysmal

Telangiectasia

The permanent changes include telangiectasia—particularly of the bridge of the nose—and pigmentation and scaling of the forearms and knees sometimes amount ing to frank pellagra Some patients have a permanently flushed face (Plate I) often with a cyanotic component whilst others have marked cyanosis of the lips and cheeks independent of any cardiac failure

Paroxysmal changes

The paroxysmal changes are even more characteristic and in the most common form the face and later the whole body flushes to a uniform and intense red Sometimes the erythema may be blotchy and closely resemble the emotionally conditioned flush of neck and chest so often seen at medical examinations Most characteristic of all but the least common is the flush originally described by Waldenstrom *et al* and consisting of areas of erythema pallor and cyanosis all intermingling in a rapidly changing kaleidoscopic pattern especially on the arms or legs An excellent illustration of this type of flush was published by Walden strom and Ljungberg (1955) Subjectively the patient may be unaware of milder attacks but more often the hands and face prickle or feel hot or become stiff or swollen and they may actually become oedematous With more

severe attacks symptoms such as sweating palpitation dyspnoea weakness nausea a sensation of pressure in the epigastrium and a feeling of tenseness may be present Changes in the blood pressure and pulse are usually slight but in very severe attacks as described by Snow *et al* (1955) and by Heimark and Parkin (1956) hypotensive collapse can occur, with intense cyanosis of the extremities and a cold wet skin The main complaint during such episodes may be of intense weakness a sense of suffocation and epigastric pain Although each flush may last only a few seconds 5 or 6 minutes is more usual but hypotensive crises may last up to 12 hours The haemodynamic changes during flushing have been studied in detail by Thorson (1956)

Precipitating factors of flushing

Although many attacks of flushing may occur without apparent reason certain precipitating factors may be recognized the most constant seem to be a meal or a drink and in one patient (Bleehen 1955) attacks occurred constantly within a few minutes of eating a fatty meal in two others (Snow *et al* 1955) invariably after minute quantities of alcohol To explain such phenomena it has been suggested that 5 HT may be released from its tumour depots in response to an intermediate hormonal mechanism akin to the action of secretin on the pancreas (*Lancet* 1955 Macfarlane *et al* 1956) Other but less constant precipitating factors may be emotional upsets and standing (Waldenstrom and Ljungberg 1955) Daugherty *et al* (1955) were able to induce flushing by palpation of a pelvic tumour deposit and by intravenous histamine but histamine had no effect in other patients and pressure on the liver (as opposed to direct pressure on a tumour mass) has not been reported as causing a flush

Abdominal symptoms

Although various abdominal symptoms have been described none of them is specific Watery diarrhoea is most common and sometimes occurs in bouts coinciding with attacks of flushing and associated with intestinal colic and borborygmi Some patients complain of epigastric discomfort described as a weight or pressure during flushing attacks Of physical signs in the abdomen ascites is fairly common and associated with enlargement of the liver due to secondary deposits which is almost invariable

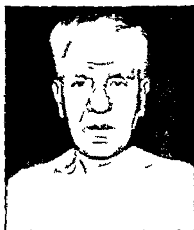
Valvular lesions of the heart

Valvular lesions of the heart have been found in about two thirds of the patients Clinically pulmonary stenosis is most commonly diagnosed but pathologically the tricuspid valve is found to be almost equally affected It is rare that signs of an aortic or mitral lesion or a combination of the two are present These patients not infrequently die of heart failure rather than of malignant cachexia

Miscellaneous features

Dyspnoea is common and in some patients will be due to cardiac decompensation It may however occur during attacks of flushing and in the absence of valvular lesions or hypertension Bronchospasm may be responsible in some patients in

PLATE I



The permanent flush of a patient with a malignant carcinoid
(By courtesy of Gastroenterologia and Sandoz Limited)

others there is no auscultatory evidence and there is a possible alternative explanation which will be discussed later

Other symptoms which have been reported include dependent oedema in the absence of congestive heart failure and severe weakness sometimes accentuated during episodes of flushing and out of proportion to the degree of cachexia which is often only slight Some or all of these features may be present in the same patient In keeping with the varying degree of malignancy of argentaffin tumours the duration of symptoms may vary from a few months to 20 or 30 years (Fraser 1955 Snow *et al* 1955) The composite clinical picture which may occur in this condition is perhaps best illustrated by quoting two case histories

In the first patient a man aged 61 years facial flushing began 9 months before he died At first attacks were provoked by alcohol but they soon occurred spontaneously and after 4 months his face was a permanent deep red colour (Plate I) by this time his liver was hard irregular and greatly enlarged Attacks continued about twice daily and in them the whole body became bright red the facial colour even more marked Each episode was associated with an urgent desire to defaecate and watery diarrhoea soon became troublesome The patient progressively lost weight and developed dependent oedema about 2 months before he died the flush began to fade as cachexia increased

The second patient a woman aged 58 years began to have watery diarrhoea 2 years before she died Soon afterwards she started to lose weight and became progressively more lethargic and breathless After 18 months of diarrhoea she noticed attacks of flushing of the hands and face 3 or 4 times a day or usually after meals and associated with palpitation Usually there was a slight flush but on one occasion transient blotches of pallor erythema and cyanosis were observed In addition to minor flushes she also experienced several severe attacks of hypotensive circulatory collapse with an almost black cyanosis of the extremities During such an attack which usually lasted several hours breathing became difficult and a sense of pressure was felt in the epigastrium A large hard irregular liver was noted about 5 months before her death but it had presumably been present for considerably longer She died after an unsuccessful attempt to resect the tumour

THE PATHOGENESIS OF THE CARCINOID SYNDROME

There is no doubt that some and perhaps all carcinoids secrete 5 HT and certain features of the syndrome may be explicable on this basis

Diarrhoea

Diarrhoea and other gastro intestinal symptoms are almost certainly due to 5 HT and not as has been suggested to any local effect of the tumour

Valvular lesions

The difficulty in breathing experienced by some patients who have neither valvular lesions nor heart failure can be due in some cases to bronchoconstriction but in others the mechanism is unknown though it may perhaps be due to pulmonary vasoconstriction which is a known effect of 5 HT

No convincing explanation of the valvular lesions has been put forward but their virtual restriction to the right side of the heart is to be noted as is the high concentration of 5 HT in venous and pulmonary arterial blood compared with its low concentration in arterial blood (Goble *et al* 1955) owing to its inactivation

by the lungs McKusick s (1956) case is particularly instructive all four valves were affected but an atrial septal defect was present presumably allowing 5 HT to short circuit the lungs Assuming that there is a relationship it is still difficult to see how 5 HT whose only known effect on the lesser circuit is to cause pulmonary hypertension could lead to gross scarring of the valves Sjoerdsma *et al* (1956b) suggested the possibility of a generalized effect of 5 HT on connective tissue as some of their patients had arthritis Asboe Hansen and Wegelius (1956) have put forward evidence in support of this view

Flushing

Although minor degrees of flushing have been observed on intravenous injection of 5 HT (Page and McCubbin 1953) nothing comparable to the intense over all flush or to the rarer cyanotic and erythematous mottling of the carcinoid patient has been produced experimentally Nevertheless Sjoerdsma *et al* (1956b) believed that the flushing is due to 5 HT and although other alternatives have to be con sidered it does seem to be the most likely cause

Pellagroid features

The genesis of pellagroid features in some patients has already been discussed Sjoerdsma *et al* (1956b) believed that a secondary tryptophane deficiency by causing hypoproteinaemia is responsible for oedema and ascites which may appear early and in the absence of heart failure The levels of plasma proteins however tend to be higher than in most types of hypoproteinaemic oedema so that some other factor may be involved

Plasma 5-hydroxytryptamine estimations

Assuming that 5 HT is mainly responsible for the carcinoid syndrome why is there no constant correlation between the secretory activity of the tumour as indicated by the output of 5 HIAA and the severity of the symptoms?

It should first be noted that the urinary excretion of 5 HIAA while reflecting the total production of 5 HT does not give any reliable indication as to the amount in circulation and particularly that free in the plasma it is however only the latter fraction which can be expected to cause symptoms Further neither serum nor whole blood estimations of 5 HT are reliable the former because of a variable release of 5 HT from the platelets and the latter because 5 HT is absorbed by the red cells from which recovery is incomplete (Hardisty and Stacey 1955) Only plasma 5 HT estimations can be considered satisfactory for correlation with symptoms In the few studies carried out in which the plasma estimations have been performed by a reliable method such as that of Hardisty and Stacey (1955) 5 HT has shown a considerable increase in the plasma suggesting that the normal mechanisms for its removal are over loaded Variations in the efficiency of these mechanisms may partly explain the lack of correlation between secretion and severity of symptoms but Davison and Sandler (1956) were unable to find an adaptive increase in lung amineoxidases

The site of the main bulk of the tumour tissue may also be important as it is notable that the majority of patients with symptoms have hepatic or pelvic secon daries which would release 5 HT almost direct into the inferior vena cava On the other hand 5 HT secreted by mesenteric deposits would be oxidized by the

liver before it reached the general circulation Another factor may be the development of tolerance for Gaddum and Hameed (1954) and Ginzel and Kottegoda (1954) have shown experimentally that tolerance is rapidly developed a patient therefore who had had a high blood 5 HT for years might have very different symptoms from one in whom the disease was of recent onset

Histamine activity

Certain other substances have to be taken into account in addition to 5 HT Pernow and Waldenström (1954) for example found large quantities of histamine in the urine of one patient with flushing attacks and a carcinoma of undetermined origin but the absence of 5 HT suggested that it may not have been an argentaffinoma Waldenstrom *et al* (1956) more recently described other patients who undoubtedly had the true carcinoid syndrome and who were excreting large amounts of histamine as well as 5 HIAA but they did not find an excess of histamine in all patients and its role is at present difficult to assess There is however the interesting question of the source of the histamine it is known that it can be liberated by 5 HT (Feldberg and Smith 1953) the presence of large quantities rather suggests that the source may be the tumour itself

Unidentified active substances

When first demonstrating the presence of 5 HT in carcinoid tumour tissue Lembeck (1953) noted that about half the activity was due to an unidentified substance other than 5 HT *not* histamine substance P acetylcholine or adrenaline Snow *et al* (1955) also found an unidentified active substance in the tumour tissue in one of their patients and Sjoerdsma *et al* (1956b) found two unidentified 5 hydroxyindoles in the tumour tissue and urine of one of their patients Some of the discrepancies in correlating the carcinoid syndrome and the known effects of 5 HT as well as accounting for the differences in individual patients may therefore be explained by the participation of histamine or other active substances in some of these cases

DIAGNOSIS

As might be expected a clinical diagnosis is usually possible but this may not be as simple as the clinical description of the fully developed syndrome might suggest owing to the misleading ways in which the symptoms may present Many full descriptions of the syndrome have been published but the presenting symptom is rarely stated

Except at the menopause in women there are few examples in clinical practice of flushing severe enough to warrant medical advice so that the diagnosis should readily come to mind if this is the presenting symptom Despite its frequency as a symptom in this condition however the patient often omits to mention it as it seems to him to have no bearing on the main complaint of diarrhoea abdominal pains weakness or dyspnoea None of these is diagnostic and unless a permanent flush is present or one is witnessed during examination this valuable diagnostic symptom may not be recognized In one patient for instance it was only at his third interview that flushing was noticed and the true diagnosis made Since flushing had been present for 20 years he very reasonably thought it irrelevant to his main complaint of abdominal pain It is not suggested that all patients with

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It should first be noted that the urinary excretion of 5 HIAA while reflecting the total production of 5 HT does not give any reliable indication as to the amount in circulation and particularly that free in the plasma it is however only the latter fraction which can be expected to cause symptoms Further neither serum nor whole blood estimations of 5 HT are reliable the former because of a variable release of 5 HT from the platelets and the latter because 5 HT is absorbed by the red cells from which recovery is incomplete (Hardisty and Stacey 1955) Only plasma 5 HT estimations can be considered satisfactory for correlation with symptoms In the few studies carried out in which the plasma estimations have been performed by a reliable method such as that of Hardisty and Stacey (1955) *5 HT has shown a considerable increase in the plasma suggesting* that the normal mechanisms for its removal are over loaded Variations in the efficiency of these mechanisms may partly explain the lack of correlation between secretion and severity of symptoms but Davison and Sandler (1956) were unable to find an adaptive increase in lung amineoxidases

The site of the main bulk of the tumour tissue may also be important as it is notable that the majority of patients with symptoms have hepatic or pelvic secon daries which would release 5 HT almost direct into the inferior vena cava On the other hand 5 HT secreted by mesenteric deposits would be oxidized *by the*

Although many antagonists are known (Woolley and Shaw, 1954 Gaddum and Hameed 1954) only a few can be used therapeutically The most potent is lysergic acid diethylamide (LSD) but this even in small doses is liable to cause mental disturbance so that its use is very limited Sjoerdsma *et al* (1956a) gave 80 micrograms to a patient with the carcinoid syndrome and the usual mental effects were produced but contrary to expectation a severe flush occurred with a sharp rise in blood pressure A second patient receiving 40 micrograms developed an attack of asthma The brominated derivative of LSD (BOL 148) stated to have almost the same potency as LSD but without the mental effects has also been used (Snow *et al* 1955 Waldenstrom and Ljungberg 1955) in doses up to 7 5 milligrams daily for 3 weeks but with little or no benefit It is possible that even higher dosage may be necessary The only other antagonist used has been ergotamine but it has been ineffective

Reserpine is said to release 5 HT from its depots (Pletscher *et al* 1955) and the administration of large doses to carcinoid patients may be dangerous However used cautiously appropriate and long-continued dosage could lead to depletion of 5 HT and relief of symptoms To the time of writing the only report of its use *and without effect has been the administration of 4 milligrams in 24 hours* (Daugherty *et al* 1955) and the author has given 1 milligram daily to one patient also without any change in the symptoms or of 5 HIAA excretion over a period of 3 months

The derivation of 5 HT from tryptophane has been mentioned and Smith *et al* (1957) found that the output of 5 HIAA could be lowered by giving a low tryptophane diet but this regime cannot be used therapeutically because of the increased liability to pellagra A low tryptophane diet with supplements of nicotinamide might be effective but the results of such a regime have not been reported

In view of the possible role of histamine in some cases antihistamines have been given but without success (Snow *et al* 1955 Waldenstrom and Ljungberg 1955)

It is doubtful if any of these forms of treatment which are mainly directed to the relief of symptoms have any effect on prognosis

The most successful therapeutic effects have been achieved by the intraperitoneal or intravenous injection of radioactive colloidal gold (^{198}Au) which becomes concentrated in the liver (Daugherty *et al* 1955 Goble *et al* 1955) or by surgical resection of large masses of the tumour tissue (Thorson *et al* 1954) When laparotomy has been performed however there have been many cases in which it was impossible to remove sufficient tumour tissue to be effective Nevertheless it is worth while to try and remove the primary growth whenever possible Otherwise the prognosis is determined by the malignancy of the tumour and can be summed up by the generalization that a short history indicates a poor prognosis and a long history a good one

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diarrhoea or other relevant symptoms should be asked if they flush for many will admit to occasional flushes emotional or menopausal some of which can closely resemble the milder flushes of the patient with a carcinoid tumour It is well to remember that hepatomegaly is the most constant clinical finding and that if absent the diagnosis is unlikely although occasional cases have been reported without liver metastases The combination of valvular lesions of the right heart with flushing and hepatomegaly is almost diagnostic

Confirmatory tests

Whatever the role of 5 HT in the carcinoid syndrome its constant presence in increased amounts may be used for confirmation of the diagnosis

Various pharmacological and chemical methods for the assay of 5 HT have been described (Robson and Keele 1956) but they are unsuitable for routine diagnostic use and Page *et al* (1955) suggested that the increased output of 5 HIAA in the urine may be a more convenient guide Normally no more than 10 milligrams of 5 HIAA is excreted in the 24 hours (Sjoerdsma and Udenfriend 1955) whereas in the carcinoid syndrome this figure may vary from approximately 40 milligrams (the lowest figure reported in patients having symptoms) to over 2 grammes So far as is known at present an increased output of 5 HIAA is diagnostic of argentaffinoma apart from occasional slight increases in other alimentary neoplasms (Clerc Bory *et al* 1954)

5 HIAA has the advantage of being relatively stable provided the urine is acidified no other precautions are needed in collection The method for the estimation of 5 HIAA used by Page *et al* and described by Udenfriend *et al* (1955) was rather cumbersome and with increasing interest in the subject the need for a more convenient technique and in particular for a rapid screening test became apparent

Simpler quantitative methods have now been evolved by Hanson and Serin (1955) and Macfarlane *et al* (1956) and the original descriptions should be consulted for detail Two qualitative methods have been described combining rapidity with extreme simplicity and consequently are of value as screening tests That described by Hanson and Serin (1955) merely consists of boiling equal parts of urine and Ehrlich s aldehyde reagent a blue colour denoting the presence of excess 5 HIAA Unfortunately interference by urinary pigments may be troublesome Curzon (1955) evolved a very simple chromatographic technique of comparable sensitivity but it takes almost 2 hours to carry out It has the advantage however of indicating the presence of 5 HT as well as 5 HIAA and occasionally this may be important In one patient for example the excretion of 5 HIAA was only slightly raised and the urine failed to give a blue colour with Ehrlich s aldehyde reagent The output of 5 HT however was greatly increased and readily detected by Curzon s method Although Hanson and Serin s method is quicker and simpler it would be unwise to give up the chromatographic technique until the relative merits of the methods have been further assessed in practice

TREATMENT

Since many of the symptoms may be due to 5 HT serotonin antagonists have been used in an attempt to alleviate them

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society was once plunged into the Dark Ages The confusion is best exemplified by still another analysis (Roberts 1950) from the same publication also within the same three week period in which the author came to the conclusion that Stress in addition to being itself and the result of itself is also the cause of itself

Most of the misunderstanding arose from the widespread practice of specious over generalization without thorough knowledge of the fundamentals of stress research On the other hand experiments by competent investigators carefully designed to test the validity of specific aspects of this theory were responsible for bringing about certain modifications and extensions of the initial concept as well as defining the areas where further gaps remained

THE GENERAL ADAPTATION SYNDROME

The concept that the organism reacts to distressing situations in terms of highly integrated metabolic activities is not new It was Cannon (1914) who first published his investigations on the adrenal medulla in which he showed that emotional stimuli were capable of releasing a substance which would prepare the animal for flight or for defence We are all familiar with such adrenergic responses which cause tachycardia increased cardiac output hyperglycaemia possibly contraction of the spleen and numerous other sympathomimetic activities such as widening of the pupils horripilation and other phenomena which might teleologically be interpreted as being purposeful reactions

It was somewhat against this background that Professor Selye first reported (Selye 1936) a series of experiments which indicated that laboratory animals exposed to a variety of widely different and even opposite noxious stimuli all responded in the same stereotyped manner This pattern of response to non specific stress was characterized by enlargement of the adrenal cortex involution of the thymus and lymphatic organs and the development of gastro-intestinal ulcers Since its nature suggested a call to arms of the body s defences it was termed the Alarm Reaction Further investigations revealed that if exposure to the specific stressor was continued a stage of resistance would occur during which increased resistance resulted in adaptation to the specific stressor and finally after prolonged exposure a stage of exhaustion would ensue during which the acquired adaptation or resistance was lost To this tripartite response was given the name the General Adaptation Syndrome Its three phases were the Alarm Reaction when adaptation had not yet been acquired the Stage of Resistance when adaptation to the specific stressor was maximum and the Stage of Exhaustion in which the acquired adaptation was useless or lost Failure of the organism to adapt successfully might occur as a result of lack of adaptational responses excessive adaptational activities or abnormal adaptational reactions Such disorders arising from failure of the organism to adapt successfully were termed Diseases of Adaptation although more properly they should be considered as disorders of maladaptation

Alarm reaction

On further study the alarm reaction appeared to comprise two sets of phe nomena those which were passive and represented signs of damage or shock

CHAPTER 21

THE GROWTH AND DEVELOPMENT OF THE STRESS CONCEPT AND ITS SIGNIFICANCE IN CLINICAL MEDICINE

PAUL J ROSCH

IT IS NOT the purpose of this chapter to analyse in detail the tremendous wealth of material that has accumulated on this subject over the past 20 years This important service has been and is being rendered in comprehensive fashion elsewhere (Selye 1950 1951 Selye and Horava 1952 1953 Selye and Heuser 1954 1955–1956) Our main effort will be to trace the salient features of the development of Professor Selye s theory and to offer an appreciation of its present value to clinical and laboratory investigators

INTRODUCTION

The desire to create order out of chaos and to make rational the obscure is a fundamental prerequisite for the scientific investigator whether his field of interest be cosmology nuclear physics or the practice of medicine Attempts to systematize and correlate the diversified phenomena comprising the subject of disease began with the Hippocratic concept of ponos but were limited for the most part to the last century the work of Virchow Pasteur and Koch being pre eminent in this regard Apart from such purely nosologic efforts theories of medicine have also centred about the response of the individual to disease and in this area the prescient investigations of Claude Bernard provided a firm foundation for all further studies particularly those of Walter Cannon

In the past two decades yet another impressive basis for a unified theory of medicine has evolved in the form of Professor Hans Selye s concept of non specific stress and adaptational syndromes As is the case with any significant departure from established modes of thinking these new theories were greeted with a range of sentiment varying from complete rejection to unqualified endorsement In many instances there was considerable misunderstanding concerning the relationship of these concepts to the discovery and utilization of adrenocorticotrophin (ACTH) and cortisone and still further confusion regarding their direct application to clinical medicine Thus an editorial in the British Medical Journal for June 17 1950 described the value of a theory as being its capacity to weld together isolated facts into a whole greater than the sum of those facts and in its capacity to stimulate research It further stated No theory in living memory has possessed these virtues to a greater extent than that of the General Adaptation Syndrome (Anonymous 1950) Three weeks later in the same journal (Meiklejohn 1950) we find the publication of Stress as perhaps serving very well the same purpose that Galen s book did in the former Middle Ages the writer cautioning that we would do well to remember that through deductive speculation civilized

✓ EXPERIMENTAL BACKGROUND

The first experiments that were performed attempted to study the effect of the stressor (cold muscular exercise and so forth) in adrenalectomized laboratory animals and this showed that in the absence of the adrenals stress could no longer produce thymicolymphatic involution as well as certain other characteristic changes On the other hand gastro-intestinal ulcers were actually more severe in adrenalectomized than in intact animals and could be lessened by treatment with certain cortical extracts It was concluded that these latter lesions were not mediated through the adrenal but were actually combated by an adequate adrenal cortical response to stress On the other hand when adrenalectomized rats were treated with the impure cortical extracts available at the time it became evident that the characteristic thymicolymphatic involution as well as other stigmas of the alarm reaction could be reproduced by these crude agents even in the absence of the adrenal It was further concluded that these changes were indirect results of stress and were mediated by certain corticoids

In 1937 an attempt was made to ascertain what stimulated the adrenal cortex during stress It was found after many surgical interventions that only hypophysectomy prevented the adrenal response during the alarm reaction Subsequent experiments demonstrated that stressors were able to stimulate the adrenal cortex via a pituitary adrenocorticotrophic substance later identified as ACTH

Later due to the efforts of Kendall and Reichstein more purified steroids became available Using desoxycorticosterone acetate (DOCA) it was possible under certain experimental conditions to reproduce in rats the characteristic evidences of damage seen in the general adaptation syndrome These included among others nephrosclerosis arthritis myocarditis hypertension and polyarteritis nodosa Yet even high doses of these compounds could not cause thymico lymphatic involution or certain blood changes characteristic of adrenal activity during stress When cortisone and other glucocorticoids became available it was evident that these were the agents responsible for the effects noted on lymphoid tissue and more important that these agents could inhibit and protect against certain manifestations of damage produced in animals by DOCA This was the first demonstration of a possible concept of adrenal checks and balances of corticoid antagonism For a more completely documented description of the development of these concepts reference should be made to a monograph of Selye (1952)

THE LOCAL ADAPTATION SYNDROME

It had been realized for some time that many local responses to topical injury were non specific and not limited to a specific agent The characteristic phenomena of inflammation might result from trauma infection chemical irritation or burns Investigation of the action of topical stressors revealed that certain similarities existed between systemic and local non specific responses Thus protracted topical stress induced a Local Adaptation Syndrome characterized by the familiar tripartite response The local alarm reaction reveals degeneration and necrosis in its shock phase acute inflammation and hyperplasia in its countershock phase This is followed by a local stage of resistance characterized by chronic

and others which appeared purposeful manifestations of active defence against damage In those examples of stress from which the organism eventually recovered the signs of injury tended to appear before the manifestations of defence phenomena and the alarm reaction could be conveniently subdivided into two separate phases a phase of shock and a phase of countershock Thus gastro intestinal ulcers appeared in the initial phase and adrenal cortical enlargement with signs of increased activity and reparative defensive actions comprised the second or *countershock phase This second phase of the alarm reaction apparently merged* imperceptibly into the stage of resistance in cases of exposure to chronic stress but its individual identity was established by showing that exposure to transient sub lethal systemic stress did not result in a state of resistance although certain phenomena of the shock phase could be reversed

Stage of resistance

The stage of resistance was characterized by increased resistance to the specific stressor to which the body had been exposed but a decreased resistance to other stimuli presented at the same time This gave rise to the concept of crossed resistance or *non specific resistance Many of the morphological* and bio chemical changes of the alarm reaction disappeared during the stage of resistance and indeed some of the effects were even reversed (deposition of lipid into the adrenal cortex)

Stage of exhaustion

The stage of exhaustion represented the composite picture of all non specific systemic reactions which inevitably developed following protracted over exposure to stressors In these instances adaptation which had initially developed could no longer be maintained It was found that even a perfectly adapted organism could not indefinitely maintain a state of complete resistance and this gave rise to the concept of adaptation energy Once adaptation was lost hallmarks of the alarm reaction reappeared such as thymicolymphatic involution gastro intestinal ulcerations and loss of adrenal lipid

The general adaptation syndrome then represented the sum of all non specific systemic reactions of the body which result from long continued exposure to chronic *stress It apparently evolved in three phases characterized by* the alarm reaction the stage of resistance and stage of exhaustion Fundamental to this concept was the realization that the manifold histological morphological biochemical and functional alterations produced by a variety of systemic stressors were essentially identical irrespective of the nature of the specific stressor Superimposed on these non specific responses could be seen the effects of specific damage elicited by the noxious stimulus but this was incidental to rather than a part of the organism s defence pattern The reaction could be elicited only by systemic stress it was a general reaction The response helped to acquire resistance it was adaptive its individual aspects were integrated and mutually dependent it was a syndrome These features were to be distinguished from specific adaptive responses such as *immunization* against yellow fever since this latter type of reaction was not evoked by systemic stress and the type of resistance produced was sharply limited to the specific stressor

presumably much like those of gonadal origin having similar effects on secondary sex characteristics and favouring protein anabolism Whether the adrenal cortex normally secretes an androgen and what the stimulus for its production is remains uncertain and raises the problem of more than one ACTH A weak androgen is elaborated during the normal process of steroidogenesis but it is then hydroxylated to cortisol Although not concerned here with this group of compounds it should be anticipated that increasing interest will centre about the possible role of this element of adrenal activity in the pathogenesis of certain degenerative disorders particularly senile osteoporosis and coronary artery disease (Starr 1956)

The generalizations made above are deceptively broad It must always be kept in mind that because of the close structural similarity of these compounds no clear-cut separation of function is expected Thus glucocorticoids have slight effects on electrolyte activity some mineralocorticoids produce repercussions in carbohydrate metabolism and other compounds have intermediate activities With the synthesis of 9 *alpha* halogenated compounds and more recently the 2 methylated analogues of these it is possible artificially to produce agents with extremely potent actions in both spheres or as in the case of the compounds with a double bond in the 1 2 position to produce actions almost only in one area This does not detract however from the usefulness of making the artificial separation

Lyophilized anterior pituitary extract

It has been noted that certain effects of stress appeared to be mediated via adrenal cortical activity and others appeared to be combated by adequate pro duction of certain hormones Reference has also been made to the apparent primary role of the pituitary in the mediation of the stress response Further experiments designed to elucidate the role of the pituitary in this chain of reactions were undertaken the principal agent utilized at this time being crude lyophilized anterior pituitary extract Such hypophyseal preparations appeared to be definitely corticotrophic in that they caused enlargement of the adrenal cortex Of con siderably greater interest was the demonstration that these extracts tended to simulate the damaging effects of mineralocorticoids especially as regards the production of nephrosclerosis When subsequent advances led to the isolation and purification of pituitary hormones it could definitely be shown that ACTH was not the agent responsible for this activity of lyophilized anterior pituitary extract On the other hand somatotrophin (STH) or growth hormone did under certain conditions reproduce nephrosclerotic changes similar to those observed with DOCA and the crude pituitary preparations

More studies indicated that STH was able to reverse or antagonize certain catabolic effects of ACTH It tended to prevent the weight loss and susceptibility to infection seen in animals heavily over dosed with ACTH or glucocorticoids For example the rat s normal resistance to tuberculosis could be overcome by pretreatment with ACTH Concomitant administration of STH would abolish this sensitivity Thus there appeared to be a similar set of checks and balances at a hypophyseal level such that STH was to ACTH as mineralocorticoids were to glucocorticoids $\left(\frac{\text{STH}}{\text{ACTH}} = \frac{\text{mineralocorticoids}}{\text{glucocorticoids}}\right)$

inflammation, hypertrophy and hyperplasia and eventually a local stage of exhaustion manifested by degeneration necrosis and atrophy ensues It was even possible to demonstrate the phenomenon of crossed resistance The possibility of interrelations between the general and local adaptational syndromes appeared likely with the realization that both were non specific defence reactions and that both evolved in three similar stages Furthermore it could be shown that both these syndromes were unusually modified by certain adrenal and pituitary hormones and that if the two responses developed simultaneously in the same organism they greatly influenced one another This was particularly true in the case of altered tissue reactivity to topical stressors in the presence of systemic stress Thus the fundamental reaction pattern to topical stressors was a local adaptation syndrome with inflammation to systemic stressors the general adaptation syndrome It was postulated that various modifications of these two basic responses might constitute the essence of certain diseases

TERMINOLOGY

The terminology which had to be adopted to describe the activity of adaptive hormones is now considered The isolation of aldosterone from the adrenal has more or less ended the unitarian conception of adrenal cortical secretion However of the various 30 or more steroids identified in the venous effluent of the adrenal to date possibly only three give evidence at present of being true hormones and they are aldosterone cortisol or hydrocortisone and corticosterone Whether the others are metabolites or precursors remains to be seen In any event from a functional viewpoint adrenal cortical secretion is most conveniently understood from a trinitarian approach This concept holds that the adrenal exerts its physiological influences in three main spheres carbohydrate metabolism electrolyte metabolism and the vague realm of sex steroids

Carbohydrate metabolism

Carbohydrate metabolism is influenced predominantly by the glucocorticoids also called glycogenic steroids or sugar hormones Examples of this group are cortisol and cortisone In addition to its effects on carbohydrates which are mediated by gluconeogenic actions and inhibition of the hexokinase reaction the glucocorticoids in general are also protein catabolic (or anti anabolic) and in addition generally possess lympholytic and eosinopenic properties More important for the purposes of this discussion is the pronounced effect that this class of compounds has on the inflammatory process and on wound healing

Electrolyte metabolism

The electrolyte controlling steroids mineralocorticoids or salt hormones were initially represented by 11 desoxycorticosterone but are now best exemplified by the naturally occurring aldosterone or electrocortin In addition to promoting the reabsorption of sodium and the excretion of potassium these compounds appear also to antagonize certain non carbohydrate activities of the glucocorticoids

The sex steroids

The sex steroids or testoids also known as N or protein hormones are

yet play a significant role in normal metabolism Small quantities have been recovered in perfusates of the gland *in vitro* (Hechter *et al* 1951) and in the dog its concentration in the adrenal vein has been found to be elevated after administration of ACTH and reduced after hypophysectomy (Farrel *et al* 1954) Of considerable interest is the first recorded report of the spontaneous occurrence of significant amounts of desoxycorticosterone in human urine (Bongiovanni 1955) This study suggested the possibility that desoxycorticosterone is normally produced as an intermediate compound in the synthesis of cortisol the conversion being facilitated by the enzyme 11 β hydroxylase If a deficiency of this enzyme exists or if it is inactivated the conversion cannot proceed This situation apparently obtains in some cases of congenital adrenal hyperplasia and it is thought that the accumulation of desoxycorticosterone is responsible for the hypertension that is seen in these cases Desoxycorticosterone may thus be a natural precursor for both cortisol and aldosterone (Wettstein *et al* 1955) a concept that has interesting implications in view of the previous experiments mentioned

The possibility of still other mineralocorticoids is raised by the isolation of 17α 19 dihydroxy 11 desoxycorticosterone from bovine adrenal perfusates (Levy and Kushinsky 1954) This compound is of particular interest in that it is more potent than DOCA in the sodium retaining test but apparently has less eosinopenic (and presumably glucocorticoid) effects than aldosterone

THE VALUE AND USE OF A THEORY

While it is not necessary for our concept to ascribe mineralocorticoid potentialities to STH itself it is of interest to note that such properties have been described and demonstrated to be operative even in the absence of the adrenal (Stein *et al* 1952) Conversely there may be found clinical (Scott and Kalz 1956) and laboratory (Menkin 1953) evidence suggesting that ACTH has local anti inflammatory properties *not mediated via the adrenal* thus completely rounding out the concept of duality and antagonism in the hypophyseal sphere

All the above observations are consonant with the general theory previously outlined but they are by no means necessary for its existence nor would their absence represent a source of serious detraction On the other hand if this theory were unable satisfactorily to embrace all the known existing facts it would obviously no longer be useful or tenable as such The reader must appreciate that we are after all dealing with a theory rather than a law and more particularly we are concerned with very broad generalities within the rather flexible framework of that theory With this in mind there are a number of complex variables which must be mentioned if only in summary fashion

Predominance of antiphlogistic or prophlogistic corticoid activity

At any peripheral target site the over all effect will depend on the predominance of antiphlogistic corticoid (AC) or prophlogistic corticoid (PC) activity This relationship is somewhat unusual in that with a fixed ratio of the two types of steroids the cortisol action predominates at high doses while the DOCA effect is most easily perceived at low levels This perhaps explains why in adrenalectomized animals desoxycorticosterone stimulates inflammatory responses while in

PROPHLOGISTIC AND ANTIPHLOGISTIC EFFECTS

The convenient subdivision into glucocorticoid and mineralocorticoid activity proved valuable also in serving to delineate other spheres of adrenal activity Thus the bulk of initial experimental evidence led to the hypothesis that anti rheumatic thymolytic eosinopenic and infection facilitating properties were linked *for the most part to glucocorticoid activity* On the other hand mineralocorticoids not only did not possess these effects but in some instances antagonized such activities This was particularly true in the case of inflammatory reactions where it could be demonstrated that glucocorticoids had strong anti inflammatory or antiphlogistic properties whereas mineralocorticoids were apparently prophlogistic in that they appeared to stimulate the proliferative reactivity or inflammatory potential of connective tissue The analogy extended further to embrace the actions of pituitary hormones in this respect and it could be shown that ACTH did indeed exert antiphlogistic effects presumably (but not necessarily) through the predominant liberation of glucocorticoids whereas STH had prophlogistic activities Thus STH was to the mineralocorticoids as ACTH was to the *glucocorticoids* $\left(\frac{\text{STH}}{\text{mineralocorticoids}} = \frac{\text{ACTH}}{\text{glucocorticoids}}\right)$

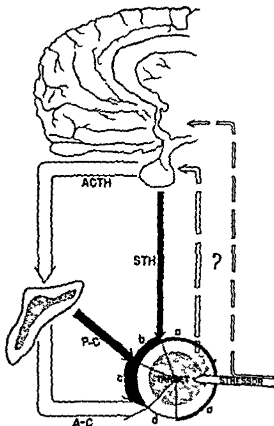
THE PROBLEM OF ALDOSTERONE

There are many gaps in the above thesis One important objection is that aldosterone has been disappointing in its prophlogistic activities as measured by its ability to counteract the anti inflammatory effects of cortisol (Selye and Heuser 1954) These studies were performed with the dosage of aldosterone calculated in terms of its equivalence to desoxycorticosterone with respect to sodium activity When compared weight for weight desoxycorticosterone and aldosterone are equally prophlogistic (Selye 1955) Using the cotton pellet implantation technique (Desaulles *et al* 1955) it was found that aldosterone had comparatively little prophlogistic activity although curiously enough in small doses it did have minimal activity in this regard This is not too surprising in view of other properties of this compound such as its effect on carbohydrate metabolism eosinopenic properties and protection afforded adrenalectomized animals against water intoxication (Gaunt *et al* 1955) In these areas it has glucocorticoid rather than mineralocorticoid effects Although it resembles DOCA more than cortisol in areas other than mineral metabolism as witnessed by its inability to restore protective influences of antihistamines against anaphylactic shock it falls short of being the prototype of the concept of an ideal mineralocorticoid because of its lack of parallel prophlogistic properties It is important to note however that it does have some effects in this direction

Significance of desoxycorticosterone

It is quite possible that aldosterone is not the final answer in the problem of endogenous mineralocorticoid excretion It has not been established unequivocally that all the sodium retaining activity of adrenal extracts is due to aldosterone or to other known steroids and the problems of isolation and extraction still offer significant obstacles There is evidence to suggest that desoxycorticosterone may

connective tissue elements as well as suppressing the normal inflammatory response Under less clearly defined conditions mineralocorticoids or prophlogistic corticoids (PC) are released such agents favouring normal connective tissue defences The mechanism of participation of mineralocorticoid or prophlogistic hormones in this phase of the response to stress requires much more investigation. It appears evident that under certain circumstances such as the stress of surgery increased amounts of aldosterone are also liberated (Llaurado 1955) The mechanisms regulating the release of STH are still less clear We have seen that the effect of this compound is to augment the natural tissue defence mechanisms but whether it accomplishes this by sensitization of the affected areas to the prophlogistic action of (PC) hormone or whether it may actually be responsible for the stimulation of production of these steroids in the adrenal cortex (Beck 1955) is not known We have adopted the former concept as the one best supported by the available evidence although both possibilities do exist



In this diagrammatic sketch the width of the circumference of the outer circle represents the existing state of local defence activities at any one moment in terms of connective tissue activity Those agents tending to augment this granulomatous potential are depicted as solid black arrows those tending to inhibit it are represented by the white arrows The normal defence potential is indicated by the width of segment (a) The effect of STH is slightly to augment this potential (b) but this occurs mainly by providing a substrate or sensitizing the affected tissue to the more profound prophlogistic effects of PC hormones which result in the maximum width at (c) However AC hormones may not only completely negate these effects but also may be sufficiently anti inflammatory to diminish even the normal tissue response as indicated by the thinness of the segment at (d) (*See* text for discussion of other factors that modify these activities)

CLINICAL CORRELATIONS

The crucial test of the general adaptation syndrome theory is the ability to find clinical corollaries to support the experimental evidence which has been presented There are a number of problems that complicate the satisfaction of this requirement and they will be discussed below It is instructive to note however that the administration of DOCA to a patient with Addison s disease apparently resulted in focal areas of necrosis in heart muscle and skeletal muscle with evidence of

the presence of the adrenal these effects are much more difficult to demonstrate It may also explain the results noted in the cotton pellet implantation experiment referred to above (Desaulles *et al* 1955)

In terms of this net result at the target site it is easily conceivable that in some instances the inflammatory response is beneficial and desirable while in others it may be deleterious Whether or not this plays a role in the determination of ultimate predominance of AC or PC activity is of teleological interest but highly conjectural It is quite likely that such factors as the nature quantity and chronicity of the stressor as well as the nature and location of the target will modify the response Perhaps the most important single factor is the phenomenon of con ditioning and this will be discussed later

Finally nothing has yet been said about the vague but obviously significant participating and co ordinating influences exerted by the central nervous system and the autonomic nervous system Equally important and equally unknown are the complex interactions of other endocrine glands which play less clearly defined roles in the over all response to stress However as previously noted the value of a theory is its ability integratively to absorb new information and it is hoped that the stress concept will be reinforced rather than compromised when such important ancillary information is obtained Fig 15 summarizes the available information concerning the potential interrelations between systemic and local reactions to a topical stressor

THE EXCEPTIONAL ROLE OF THE KIDNEY

The kidney possesses rather unusual properties that distinguish it from other targets of corticoid activity It has been noted previously that nephrosclerosis may be produced by desoxycorticosterone under certain conditions If cortisol is given simultaneously this phenomenon is aggravated rather than prevented or ameliorated Hence in this instance there is no glucocorticoid mineralocorticoid antagonism but rather a synergism A similar synergism exists in the therapy of adrenal insufficiency Of further interest is the observation that the nephrotoxic effects of STH (and methylandrostenediol) are prevented by adrenalectomy although other properties (for example prophlogistic activities) are not

Fig 15 —The stressor penetrates the normal defences (a) to reach the target Damage to the target produces local inflammation and if sufficiently severe degeneration and necrosis This appears to stimulate a variety of local factors which tend to repair the damage Through some unknown pathway (represented by ?) a stimulus travels from the injured target directly to the anterior pituitary The nature of this first mediator of hormonal defence is poorly understood It conceivably may differ according to the nature of the target or the characteristics of the stressor In some instances it may be represented by a discharge of adrenaline in others it may be a neural or neurohumoral stimulus Such possibilities include the release of histamine like substances or tissue metabolites at the site of injury On the other hand the chain reaction may be set off by a local transitory *deficiency of some vital component of cellular metabolism (enzyme* catalyst vitamin glucose) Indeed any combination of factors mentioned may be operative As indicated in the diagram the stressor may in addition act directly via hypothalamic hypophyseal pathways In any event stimulation of the anterior pituitary leads to an increased output of ACTH and the resultant liberation of glucocorticoid or antiphlogistic corticoid (AC) hormones such as cortisol and cortisone These agents inhibit the ability of the target to put up granulomatous barriers and in certain situations cause involution of

polyarteritis so that it merely blocks or conditions the conditioning agent The reader will surely appreciate the complexity and futility of extending this type of observation further

Individual stressor reaction

The problem of conditioning offers one of the greatest obstacles and challenges to the attempt at applying the precepts of stress research to clinical medicine Here the phenomena of conditioning may prove to be more important than any other factor in determining the quality (type) as well as quantity (degree) of the response The problem is conveniently illustrated in the following Table

TABLE

INDIVIDUAL STRESSOR REACTION

ECTOMORPH	*Tends to react to situations by cerebration* Serious Intellectual Enjoys reading Tends to be introverted	Cerebrotonic
ENDOMORPH	*Tends to react to situations in terms of the gastro-intestinal tract* Jovial Good natured Enjoys sensual pleasures Tends to be extroverted	Viscerotonic
MESOMORPH	*Tends to react to situations in terms of physical activities* Competitive Aggressive Enjoys athletics Tends to be ambiverted	Musculoskeletotonic

Let us assume that we have three individuals of the same age and sex and apparently similar in all respects except their body type One is an ectomorph one an endomorph and the other a mesomorph Psychiatrists and students of somatology tell us that body type has considerable influence on or correlates well with general affect and reactions to situations Thus ectomorphs tend to be introverts they are usually tall slender individuals and tend to be serious to enjoy reading and to be cerebrotonic The endomorph a round individual is usually jovial good natured extroverted and enjoys sensual rather than intellectual pleasures he is said to be viscerotonic The mesomorph is the well developed athletic type very muscular and strong delighting in physical prowess and allegedly musculoskeletotonic

Theoretically if each of these men were approached by a bandit and ordered to forfeit his money or his life the ectomorph being cerebrotonic would try to talk his way out of the situation perhaps by reasoning with the bandit and persuading him that he was taking a great risk and would not get much money anyway The endomorph being viscerotonic would tend to express himself via his gastro intestinal system and possibly respond to the situation with nausea vomiting abdominal pain or more commonly diarrhoea The mesomorph expressing

polyarteritis nodosa (Thompson 1950) In another patient with this disease vascular changes in the kidney resembling nephrosclerosis were attributed to DOCA administration (Deamer and Silver 1950) Intensification of arthritis in patients with Addison s disease who were receiving DOCA has recently been reviewed (Kirkeby 1954) and the rapid development of incapacitating arthritis following the administration of DOCA was also noted The tendency to develop hypertension on otherwise minimal or moderate doses of DOCA is a not uncommon problem in the experience of any clinician who has handled a number of cases of Addison s disease There are other examples which might be cited and conversely there is a considerable lack of evidence but on the whole clinical findings to date though meagre tend to be explicable on the basis of certain broad principles of the theory

CONDITIONING

Subjection to stress

Reference has already been made to the phenomenon of conditioning and its critical role in the interpretation of the stress concept has been mentioned Conditioning as viewed here may be taken to comprise any factor which modifies the target organ s response to corticoid action or which alters the corticoid response to stress This obviously covers a wide field and includes such diverse factors as age nutrition and heredity as well as previous treatment with corticoids Probably the most important conditioning factor is previous subjection to stress which conceivably might affect both the reactivity of a target organ and the release of adaptive hormones The definition given above is perhaps not a true representation of its usual meaning in stress research since the term as employed by Professor Selye has the connotation of sensitization or desensitization and *implies that the* conditioning activity modifies the response in terms of quantity only As will be developed later it is conceivable that the ability to cause altered or qualitatively different responses should also be anticipated and reserved for this activity *

Cardiovascular and renal damage

The most familiar example of conditioning action is the enhancement of the development of cardiovascular and renal damage by unilateral nephrectomy and salt loading in the rat prior *to treatment with DOCA Under these conditions* hypertension readily ensues although without such measures chronic or prolonged treatment with DOCA is necessary to get the desired effect A more complicated extension of this conditioning phenomenon is afforded by considering the additional effect of thyroidectomy in the unilaterally nephrectomized salt loaded DOCA treated rat (Salgado 1954) When this procedure is performed there is complete protection against hypertension as well as against the myocardial and renal lesions caused by DOCA but oddly enough the incidence of mesenteric polyarteritis is exaggerated It is supposed that this represents a dissociative conditioning of the conditioning agent Thiouracil has similar effects but does not cause increased

* In fairness to Professor Selye it must be admitted that when his concept is viewed in terms of the reaction theory it becomes apparent that in any mosaic of actions selective conditioning will bring about phenomena which impress us as being qualitatively different from the unconditioned response (for example increasing the yellow component in a blue dye may result in a green colour)

stress require the presence of corticoids but are not dependent upon increased adrenal cortical secretion for their existence In this sense the adrenal does not cause the effect but merely permits it to occur by maintaining normal or increased tissue reactivity Restated in another way adrenal hormones condition the reaction of the target to the stressor Such an explanation would equally well explain the results noted in Thorn s experiment A further extension of this proposition holds that the increased adrenal secretion during stress serves to maintain homeostasis rather than cause hypercorticoidism The increase in corticoids is occasioned by the increased need of the tissues for the corticoids At this level therefore a state of eucorticoidism or hypocorticoidism may exist due to the balance existing between availability and utilization

Steroid diabetes

The leading proponent of the concept of the permissive action of hormones is Ingle and he has recently reviewed his position on this subject (Ingle 1956) A particularly important experiment concerns the phenomenon of steroid diabetes due to 11 oxygenated steroids The point is made that it is difficult to exacerbate experimental diabetes by non specific stress Many stressors caused a decrease in the severity of experimental pancreatoprivic diabetes as determined by reduction in glycosuria and when the diabetic adrenalectomized rat treated with mainten ance doses of cortical extract is exposed to severe stress the glycosuria decreases as though the animal has now become adrenally insufficient (Ingle and Nezamis 1950 Ingle 1951) The demonstration by Engel (1952) that the catabolic response to stress occurs before the response to corticotrophin or to glucocorticoids and the notation by Venning (1955) that in general increased aldosterone elimination begins only several days after trauma whereas electrolyte alterations suggestive of mineralocorticoid activity occur almost immediately (Moore 1954) also suggests that certain adrenal responses to stress require but are not caused by adrenal activity

There are certain arguments that might be offered to these examples Although it is common practice one wonders if in this instance it is justifiable to make the assumption that adrenal cortical activity can be effectively estimated by such easily influenced and metabolically remote criteria as the level of the blood sugar much less glycosuria Another assumption which is made is that the two forms of diabetes are additive so that having a state of regulated pancreatoprivic diabetes the addition of steroids (stress) would invariably cause an exacerbation The mechanism of steroid diabetes is apparently different in certain important respects from that of diabetes mellitus particularly with respect to alterations in pyruvate metabolism (Frawley 1955a 1955b) If partial pancreatectomy interfered with the mechanism of steroid diabetes in some way this experiment would have to be re examined although evidence to support this is quite remote (Knick 1954) Certainly alloxan and phloridzin diabetes are not equatable If one wished to extend the argument further it might be postulated that evidence exists that stress may cause hyperglycaemia in part via increased glucagon excretion (Fedeli and Jelmoni 1955) In this eventuality pancreatectomy would inhibit such gluco corticoid manifestations of stress and the increased need of the tissues for energy in the form of carbohydrate would decrease the glycosuria Obviously the specific effects of the stressor used might also play a determining role in the amount of

himself via his musculoskeletal system would theoretically punch the fellow in the jaw or run as fast as he could

In this admittedly made to order illustration we would have three individuals similar in all respects save in their body build (conditioning factor) exposed to the same qualitative and quantitative stressor reacting in three entirely different ways Obviously *few persons fall wholly into one of these categories* and being combinations of each the responses would be much more complex and difficult to anticipate that is if the relationship between reaction pattern and body type is valid It is equally clear that many other factors would control the response and the difficulty in ascribing priority to any one conditioning factor is readily seen

It may be argued that the example cited has no endocrinological implications and that it describes purely behavioural reactions to a specific stressor Nevertheless it may be wondered if in these three individuals the eosinophils all dropped the same amount whether the blood sugar response was identical and whether one individual might not have a rise in blood pressure when another was unaffected or going into shock The point is raised merely to indicate the tremendously important role yet undefined of nervous mechanisms in integrating and modifying the response to stress and to illustrate the complex variables that are covered by the term conditioning

CONDITIONING VERSUS PERMISSIVE ACTION OF HORMONES

Stress or corticoids as conditioning agents

The problem of conditioning reaches its *ultima Thule* when one is forced to consider stress or corticoids as conditioning agents It is necessary to have a clear understanding of terms since this subject has aroused much controversy and misunderstanding As has been noted derangements are thought to occur during the general adaptation syndrome as a result of quantitative disturbances of adrenal response or by alterations in the balance of glucocorticoid versus mineralocorticoid If the situation is examined further it is readily apparent that the determination of whether excess hormone of either type is present at the target site is a function not only of the amount of hormone produced but of less tangible factors such as the need of the peripheral target for the steroid in question the rate of utilization the rate of detoxication and our ubiquitous companion conditioning

It seems quite likely that in addition to its ability to increase corticoid production stress also affects certain peripheral targets by increasing their sensitivity to corticoids a conditioning effect which was first recognized clinically by Thorn *et al* (1955) These investigators found that only apparently in severe stress could an actual excess of cortisol like activity be demonstrated but that even mild stressors could elicit characteristic manifestations of glucocorticoid response without demonstrable evidence of increased hormone secretion Thus it might be argued that stress apparently sensitized or conditioned the peripheral target to the action of glucocorticoids producing relative hypercorticoidism

Role of the adrenal

An alternate and equally tenable hypothesis is the suggestion that the role of the adrenal in the development of alterations following stress is a more or less passive one In other words certain metabolic consequences of the response to

the adrenal) and further suggests that certain manifestations of the stress response require no corticoid permissibility or adrenal participation

Thus many theoretical possibilities can be conceived of interaction between stress and the adrenal (1) non specific effect due to over production of corticoids (2) non specific effect due to decreased elimination of corticoids (3) non specific effect due to conditioning of the target by corticoid activity causing increased sensitivity (4) non specific effect due to the stressor but requiring the presence of corticoids to maintain tissue reactivity (5) specific effects due to the stressor itself and not influenced by adrenal factors (6) specific effects due to the stressor itself requiring adrenal activity and (7) any combination of the above

In the author s opinion there is no irreconcilable conflict between the concepts of conditioning and permissive action However while it is interesting and valuable to speculate about the possible modes of hormonal participation it is more important to keep sight of basic principles and to avoid not seeing the forest for the trees We must not be preoccupied with deciding whether to view these activities of the adrenal cortex as conditioning (Selye) permissive (Ingle) homeokinetogenic (Conn) or providing grease for the metabolic axle wheel (Engel) The important contribution is the observation that the adrenal has anything to do at all with non endocrine disease

PRIORITY VERSUS AUTHORITY

Inevitably in discussions of this sort the spectre of priority looms up and confuses the issue by injecting personalities and by associating temporal priority with authority To set the record straight the relation between hypophysectomy and adrenal cortical activity was noted in amphibia 40 years ago (Allen 1917 Smith 1916) and the ability to correct this defect with pituitary extract was described shortly afterwards (Smith and Smith 1923) Comparative results in mammals were reported over 30 years ago (Smith 1927) Partial separation of ACTH from other anterior pituitary hormones was accomplished by Collip *et al* (1933) Experiments demonstrating that adrenal cortical hyperplasia followed stress and could be suppressed by administration of adrenal cortical extract were performed by Ingle over 20 years ago and reported shortly afterwards (Ingle 1938) The sedulous investigator will even find the term stress used in describing the essentially adrenal character of shell shock and war neuroses 40 years ago (Harrower 1916 1929) Such observations often lead to polemics which prove nothing They tend to confuse the issue by raising the question of *who* is right rather than *what* is right The basic materials were certainly present in 1936 before the initial concept of the general adaptation syndrome was stated Again the important contribution has been the attempt to integrate existing factual matter and the demonstration that the adrenal could be involved in some way with non endocrine disorders and adaptive processes

An unfortunate criticism of the stress concept is that it has attempted to claim credit for the discovery and use of ACTH and cortisone (Meiklejohn 1950 Rosenberg *et al* 1952) Nothing could be further from the truth The author can state from personal experience that Professor Selye has disavowed any responsibility for or influence on the development of these agents Moreover he has stated (Selye 1951) Actually the reverse is true It is I who throughout the years

glycosuria due either to direct extra adrenal action on the blood sugar or renal threshold Unwarranted assumptions and criticisms such as those offered do not detract from the important and significant implications of Ingle s observations but they do emphasize the need for more refined criteria of the response to stress

Wound healing

There is other evidence against a purely permissive role for the adrenal during stress It is known that non specific stress elicited by traumatic surgery tends to inhibit wound healing In adrenalectomized rats maintained on small amounts of adrenal cortical extract the stress of traumatic surgery does not result in a delay in wound healing (Chassin *et al* 1954) In this instance at least increased adrenal activity does not serve to maintain homeostasis but rather to cause hypercorticoid ism A similar argument which may be offered is that the criteria used to denote the effects of stress are those characteristic of corticoid activity If the adrenal merely played a supporting role it might be suspected that the effects noted would be those of the stressor rather than the adrenal cortex

7 hydroxycorticoid concentrations

Recent studies of free plasma 17 hydroxycorticoid concentrations in adrenalec tomized dogs maintained on cortisol suggest alternative possibilities (Steenburg and Ganong 1955) In these experiments the test animals were exposed to immobiliza tion anaesthesia and surgery All were adrenalectomized and all were given identical infusions of cortisol The plasma level of corticoids was significantly higher in the anaesthetized and operated animals than in the conscious immobilized ones The logical conclusion would appear to be that surgical trauma can activate a mechanism which delays the disappearance of free 17 hydroxycorticoids from the circulation

Hepatic damage

There is definite morphological evidence of hepatic damage during the alarm reaction (Selye 1950) and in rats exposed to a variety of stressors (formalin cold spinal cord transection) there is marked reduction in hepatic function as measured by the bromsulphthalein test (Mann and Lemonde 1951) In patients with liver disease the rate of disappearance of intravenous cortisol is inversely proportional to the degree of hepatic damage measured by the bromsulphthalein test (Brown *et al* 1954) although tetrahydrocortisone disappears at an independent rate These and other studies support the suggestion that impaired hepatic detoxication of corticoids may participate in the hypercorticoidaemia caused by trauma (Tyler *et al* 1954)

Eosinopenia

Eosinophil counts during the experiment noted by Steenburg and Ganong (1955) demonstrated an interesting paradox The most profound eosinopenia occurred when the animals were immobilized and when blood corticoid peaks were lowest A further drop was demonstrated in three adrenalectomized animals which were operated upon without replacement hormone and without a demon strable rise in the circulating corticoid level This indicates that the eosinophil is sensitive to substances other than those of adrenal origin (at least in the absence of

Another pitfall is the temptation to employ a sort of *post hoc ergo propter hoc* type of argument Thus the demonstration that corticoids benefit rheumatoid arthritis is not sufficient evidence in itself to conclude that rheumatoid arthritis is due to a local deficiency of corticoid any more than it could be due to a local deficiency of aspirin or phenylbutazone Accompanied by other pertinent evidence it is a valuable observation and might support such a hypothesis This type of reasoning may often be used efficaciously as in the diagnosis and treatment of mild hypothyroidism by therapeutic trial It is important to remember however that a potential fallacy in the basic hypothesis exists Serious students of stress will avoid these pitfalls

ANTELOPES AND ADAPTATION

Mindful of the pitfalls of analogy one still notes that teleological concepts are always the most appealing and that the basic supposition of the general adaptation syndrome finds many supporting analogies in Nature Let us consider for example the adaptational problems posed by evolution as in the case of the antelope

We comprehend that an essential feature of the adaptation of antelopes is the development of horns It is their principal means of offence in struggles within their group and of defence against attack from other animals All the 23 varieties of antelope in the Belgian Congo possess horns yet they are otherwise quite dissimilar (Schouteden 1947) There must be one type of horn which is basically the most efficient and with minor variations in proportion or shape depending on the size and habits of the animal Yet it is clear that none of these antelopes possess this best horn Some horns are too small to be effective (duiker) others are prohibitively unwieldy (kudu) The horns of the impala with their double curve achieve the same placement and direction as those of the reedbuck which has a single curve but they are much weaker mechanically and without affording any conceivable advantage

If evolution were operating under a fixed plan such radical discrepancies would not occur On the other hand there is a definite orientating factor since all the animals appear to have horns which serve them adequately if imperfectly It appears that different mutations have occurred in different lines and that as long as they have served the adaptive end that is the development of a functional horn they have been retained with ancillary disadvantages This illustrates the principle of opportunism in evolution (Simpson 1949) which is the ability to meet a need with what is available even if it is not the best available and even if it is harmful in other ways One cannot subscribe to the finalist or vitalistic theory that the changes which arise are the best ones needed for adaptation but conversely if there were no orientating factor at all and the horns developed purely by chance then we should have bizarre results and ineffectiveness We have seen that however imperfect the horns are useful

Perhaps adaptation to stress resembles adaptation in evolution The adaptational requirement imposed by stress is the restoration of homeostasis in this case the development of effective horns The requirement is satisfied by using the best means at hand though often these are not the most efficient means bad side-effects or diseases of adaptation may result for example unwieldy and burdensome horns One could not assume that the over all result was the best

have been stimulated and aided by the investigations of those who have prepared corticoids and ACTH and again From all this I hope that it will be perfectly clear now that it was not the work of Hench that was inspired by the observations of the Montreal group but it is our work that receives its greatest inspiration from theirs One could hardly misconstrue this as a claim for priority

PITFALLS

One must always anticipate the danger of generalizations extending far beyond the support of actual data which they intend to generalize There is a natural tendency for any theorist to attempt to fit more into his conceptual framework than it can possibly contain The application of the concept of cybernetics to the complicated intricacies of the human brain is an example of this From an epistemological point of view *it may well be impossible to solve the problems* which have been posed

One pitfall in the use of the general adaptation syndrome is the attempt to use it as a rigid law rather than as a theory It is not possible in the present state of knowledge to account for the aetiology of all disease processes and even among those we think we understand in terms of this concept the endocrine response to stress may be only one of several modifying factors Thus while adrenalectomy *and hypophysectomy modify the stress response they do not totally prevent it* Similarly the stage of resistance can be produced in adrenalectomized rats if they are exposed to stressors gradually It has already been noted that under certain circumstances eosinopenia following stress may not be mediated through the adrenal

In many instances the problem of over generalization arises from the over extension of an analogy To illustrate we note that cortisol inhibits the inflam *matory response or is antiphlogistic As supporting evidence accumulates* we generalize and say that probably glucocorticoids in general are antiphlogistic We note that DOCA and some mineralocorticoids are prophlogistic and that they antagonize the actions of cortisol in this respect Hence perhaps all or most other mineralocorticoids are also prophlogistic These suppositions have proved to be useful and are in accord with existing facts They serve as a scaffold on which to form other deductions but if conflicting material is discovered the scaffolding has to be torn down and remodelled for it no longer has firm foundations

May we on the basis of the facts noted above assume that glucocorticoids and mineralocorticoids will always be antagonistic? The answer is obviously in the negative since we know that the activities of DOCA may be complementary and synergistic in the treatment of Addison s disease as well as in the experimental production of nephrosclerosis In this instance we would have been guilty of pushing the analogy too far Instead of using it as a sort of metaphor to describe one phenomenon in terms of another quite distinct phenomenon we have made the dangerous assumption that the phenomena really are the same sort of thing and that we can find in one an equivalent with all the features of the other This is the defect of all analogies we may come to think that they will help us to interpret and even explain phenomena when in fact they only help imperfectly and often unrealistically This is not to say that analogies are not helpful and useful but they should only extend so far as they are supported by existing facts

responses to stress and that certain disorders may result from inappropriate by products of these responses Moreover it is responsible for the demonstration that the adrenal may be involved in the production or maintenance of certain non endocrine disorders and it attempts to define those areas in which stress therapy is likely to be most effective

In the author s opinion its greatest contribution cannot be assessed at this stage It resides in its heuristic merit its ability to cause its proponent and his colleagues to strive to seek to find and not to yield

No theory could ask for more

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possible but conversely one could not conceive that the end results happened by chance since they are reproducible and if there were no adaptation to stress the *antelopes would succumb immediately since there would be no homeostasis in* the guise of effective horns The analogy cannot be pushed too far but numerous other examples could be cited to illustrate that Nature frequently hyperreacts to a stimulus by an exaggerated or inappropriate response which is potentially harm ful that is hyperplasia followed by malignancy scar tissue and keloids

CONCLUSIONS

What then can we say of the present value and limitations of the concept of stress and adaptive disorders? Certain important principles have to be kept in mind One of the most important and least respected is the realization that the response to stressers is by definition a non specific reaction It is not a disease state but in its ideal form a physiological response to injury which has as its main purpose the preservation of vitality and the maintenance of the constancy of the *milieu interieur* This interpretation must be differentiated from that in more widespread usage and denoting damage or injury The concept of Pasteur and Koch postulated that disease was caused by specific pathogens and that the organism responded by specific adaptive reactions The therapeutic implications were obvious and based on the principle of complementing and imitating such reactions when they were deficient

The general adaptation syndrome is in one sense the antithesis of this It holds that many diseases have no single cause and no single pathogen that certain *disorders are largely due to non specific stress and exaggerated or inappropriate* responses which are by products of otherwise physiological adaptive processes It implies also that antistress therapy ideally should attempt to complement the individual s own purposeful adaptive pharmacological efforts and so emulate the *vis medicatrix naturae* Further by the understanding of the *modus operandi* of disorders of adaptive failure it might conceivably be helpful to utilize those agents which would oppose the specific hormones which played a role in their patho genesis even if Nature did not use such a mechanism

It is too early to be able to offer an adequate appreciation of the concept of stress and the general adaptation syndrome Its implications are staggering and in the words of a notable authority If it is true it will represent the greatest advance in the understanding of diseases since the introduction of the germ theory (Ingle 1951) As emphasized previously interpretation of human disease in terms of animal experimentation is likely to be fraught with danger this is particularly so in the field of stress

It is difficult to understand something unless you can measure it The tests used to evaluate adrenal function are still rather crude and those employed to measure stress are certainly less than adequate Thoreau in paraphrasing Confucius once wrote To know that we know what we know and that we do not know what we do not know that is true knowledge The second half of this aphorism is most applicable to our thesis

The concept as it stands certainly offers more than a series of ingenious pharma cological exercises It provides a firm framework for educated speculation based on the premise that hypophyseal adrenal pathways are involved in the

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